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# Translation

## SOVIET SCIENCE AND TECHNOLOGY POLICY

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# SOVIET SCIENCE AND TECHNOLOGY POLICY

This non-serial report contains selected translations of Russian articles on the planning and administration of Soviet research and development and the introduction of scientific achievements into industry.

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# MARCHUK DISCUSSES MOSCOW'S TECHNOLOGICAL RESEARCH

Moscow MOSKOVSKAYA PRAVDA in Russian 11 Jul 81 p 2

/Article by G. Marchuk, deputy chairman of the USSR Council of Ministers, chairman of the USSR State Committee for Science and Technology, academician: "Efficiency Plus Saving"/

/Text/ Moscow is a true center of the country's scientific and technical progress. The headquarters of Soviet science--the USSR Academy of Sciences, academies of medical and pedagogical sciences and the All-Union Academy of Agricultural Sciences imeni Lenin--is located there. Hundreds of scientific research and planning institutes and design offices and dozens of higher educational institutions are concentrated in the city. Hundreds of thousands of people work in scientific institutions. They perform a significant part of all the research conducted in the country. In the capital there are about 40 scientific production associations. To utilize this vast potential efficiently and soundly during the 11th Five-Year Plan means to make a weighty contribution to the realization of the historical decisions of the 26th party congress.

The collectives of a number of Moscow enterprises took the initiative to develop a movement for a wide introduction of the achievements of science and technology into production, which make it possible to ensure the maximum saving of labor, material and power resources. This initiative was approved by the CPSU Central Committee. I would like to discuss some problems connected with the development of this valuable undertaking.

A successful development of scientific and technical progress depends on the coordinated work of scientific institutions, scientific production and production associations and higher educational institutions. The outstanding achievements of Soviet science and technology in the area of development of atomic power engineering, in the conquest of outer space, in the control of widespread diseases and in the creation of electronic, microbiological and other new sectors of industry are widely known all over the world.

I would like to stress that these and many other achievements began with fundamental scientific research. Important fundamental scientific ideas open up new paths for the development of science and determine the prospects for scientific and technical progress. We are rightfully proud of the results of work of scientists at the Moscow institutes of the USSR Academy of Sciences, who not only made an outstanding contribution to the knowledge of laws governing the material world, to the investigation of the universe and to the study of the processes of social development, but also did a great deal for the solution of important national economic problems.

We must continue to see to it in every possible way that the attained high level of fundamental and applied research of academic institutes is retained, strengthen and expand their experimental base and take measures to accelerate the introduction of their achievements into practice. Sectorial scientific research institutes and industrial enterprises in Moscow should actively participate in the realization of these achievements. At the same time, it is necessary to proceed from the instruction of Comrade Leonid Il'ich Brezhnev, who stated the following at the 26th party congress: "The country is badly in need of a greater concentration of the efforts of 'large-scale science,' along with the elaboration of theoretical problems, on the solution of key national economic problems and on discoveries capable of introducing truly revolutionary changes into production."

Science in higher schools now has great potentials. Having available highly skilled professorial and teaching personnel, many higher educational institutions in Moscow are recognized centers of scientific thought and make an important contribution to the development of science and the national economy.

Such leading higher educational institutions of our country as Moscow State University, the Moscow Order of Lenin and Order of the Red Banner of Labor Higher Technical School imeni Bauman, the Moscow Engineering Physics Institute, the Moscow Power Institute, the Moscow Aviation Institute, the Institute of Steel and Alloys and many others can be mentioned.

Moscow State University is rightfully the flagship of our higher schools. Its collective of many thousands of people conducts scientific research in almost all branches of modern knowledge. The results of 400 completed scientific investigations were introduced into the country's national economy in 1980 alone. The economic benefit totaled 25 million rubles. The capital's enterprises, with which the university fruitfully cooperates, include the Kauchuk Plant, the Moscow Order of the Red Banner of Labor Automobile Plant imeni Leninist Komsomol, the Stroyplastmass Association, the Factory imeni Sverdlov and the Moscow Water Supply Trust.

Unfortunately, however, there are also higher educational institutions where efficiency is still low, the gain from the introduction of research results hardly covers the expenditures on them and the transfer of research results to industry extends over many years. Unsuccessful scientific establishments are still encountered in higher educational institutions, including among scientific research problem laboratories performing secondary and nonurgent work.

State bodies and sectorial ministries and departments must intensify their attention to problems of financing and material and technical supply for research conducted in higher educational institutions. Most higher educational institutions cannot place their work at the proper level owing to the lack of the necessary laboratory, experimental and production and technical base and, as a rule, national economic enterprises are not involved in the training of their personnel right up to the entry of young specialists into employment. It is necessary to more widely and boldly utilize enterprises, scientific research institutes and design offices as the base for training students in higher courses.

There is another problem. More than 30,000 graduate students study in the capital. However, we do not always train highly skilled specialists in the trends needed, for example, laser engineering and plasma processes. Thus, only eight candidates of sciences are being trained in powder metallurgy this year! At the same time, the number of specialists in traditional fields turned out is 1.5 times as high as needed.

The largest number of scientists and specialists is concentrated in sectorial scientific research institutes and design offices in the capital. Such leading scientific research organizations as the Institute of Atomic Energy imeni Kurchatov, the Central Scientific Research Institute of Heavy Machine Building, the All-Union Scientific Research and Planning-Design Institute of Metallurgical Machine Building, the All-Union Electrical Engineering Institute imeni Lenin, the Central Institute of Aircraft Engines, the All-Union Scientific Research Institute of Light Alloys, the State Scientific Research and Planning Institute of the Rare Metals Industry, the Physicochemical Institute imeni Karpov and many others are widely known for their advances.

The cooperation of the All-Union Scientific Research and Planning-Design Institute of Metallurgical Machine Building, the Central Scientific Research Institute of Automobiles and Automobile Engines, the Tulachermet Scientific Production Association, the Scientific Research Institute of Technology of the Automobile Industry and others with the AvtoZIL Association, in which the institutes of the USSR Academy of Sciences and of the Ukrainian Academy of Sciences also participate, is a striking example of the great contribution of sectorial scientific research institutes to scientific and technical progress. The plan of this cooperation contains about 300 topics. Laser welding, new technology of pinion rolling, control automation and many other developments ensure a high technical level of production and quality of output.

Sectorial and academic institutes and higher educational institutions in Moscow participate in the cooperation with production associations and enterprises on the basis of the formed scientific research trend and the capabilities of the experimental and pilot base; in brief, with due regard for their own specific features. However, there are many common fairly important problems for all the enumerated scientific institutions. They should jointly solve the problems set by the 26th party congress.

First of all, this involves the maximum possible saving of labor resources, which is especially urgent for Moscow. Mechanization and automation are the main trend in the labor saving scientific and technical policy. Extensive work on the mechanization and automation of production is done at many enterprises in the capital, including at the Plant imeni Vladimir Il'ich, the Krasnyy Proletariy Plant, the Plant imeni S. Ordzhonikidze, the Borets Plant, the Kompressor Plant and so forth. Nevertheless, on the whole, the problem of reducing manual labor at the capital's enterprises still remains acute and requires additional efforts.

Meanwhile, the analysis made by the State Committee for Science and Technology with the participation of specialists and scientists shows that an extensive utilization of the mechanization and automation equipment already developed and mastered in production makes it possible to reduce the number of workers engaged in manual labor to one-fourth. The experience of advanced Moscow plants confirms the reality of attainment of such indicators.

Unfortunately, however, as of now the volume of production of manipulator robots does not meet the increased demands of the national economy, including the needs of Moscow's enterprises. Therefore, thought should be given to the advisability for the specialization of one of Moscow's major enterprises in the output of this equipment, especially as many of the city's scientific research institutes, higher educational institutions and enterprises participate in the all-Union program of work on manipulator robots.

It seems advisable to record work places at Moscow's enterprises for the purpose of uncovering operations at which manual labor is used and to determine the list of equipment, including automatic manipulators, making it possible to replace manual labor with machine labor at these operations.

Apparently, it is necessary to entrust the city's leading technological organizations with the modernization of existing and the development of fundamentally new technological processes with the use of manipulators. The organization on a cooperative basis of the production of models of automatic manipulators with program control, as well as of the accessories for them, necessary for Moscow enterprises can be of great benefit.

The use of processing centers of multipurpose automatic machine tools with program control in combination with manipulator robots is a new tendency in the development of automatic production facilities. To ensure such a high level of automation, it is necessary to widely use computer equipment. More than 90 Moscow organizations and enterprises and many higher educational institutions in the capital participate in this work.

It should be noted, however, that automated systems for controlling technological processes are introduced at insufficient rates in Moscow. Only 39 systems, or less than 3 percent of the total number of the automated systems for controlling technological processes introduced in the national economy, were put into operation during the past five-year plan. Ministries and departments should analyze the possibility of using such systems at Moscow enterprises and draw up a plan for their introduction during the 11th Five-Year Plan. It seems to us that party organizations should place these problems under special control.

The automation of planning and designing is no less important. After all, in Moscow there are hundreds of major planning and design organizations, in which collectives of many thousands of people work. An increase in the productivity of their labor and the disengagement of part of the workers represent a vast potential during the 11th and subsequent five-year plans. For example, the use of the system of automated planning in the All-Union Scientific Research Institute of Electromechanics made it possible to accelerate the development of asynchronous electric motors five- to six-fold. As a result of the optimization of design solutions an annual saving of about 1,000 tons of electrotechnical materials is attained and 150 million kilowatt hours of electric power are saved annually during the operation of electric motors.

Finally, we must not fail to mention the automation of scientific research. This trend increases the labor productivity of scientific workers three- to five-fold, speeds up research and produces a great national economic effect. However, it is impossible to establish automation systems without personnel familiar with modern methods.

A few words should also be said about automated production control systems. The capital is a planning and scientific center for control automation. An overall object program for the establishment and development of interconnected automated systems for controlling the city economy for the 11th Five-Year Plan and for the period until 1990 was examined and approved at the joint meeting of the board of the State Committee for Science and Technology and the executive committee of the Moscow City Soviet in March of this year. Provision was made for an accelerated establishment of computer centers for collective use, primarily in construction, public health, transport, trade and public dining, which are to be unified into a single computer network by means of a data transmission system.

This is extremely necessary, because the number of computer centers in Moscow, that is, 742, has become intolerably large. More than 2,000 computers operate in these computer centers. They are serviced by a large personnel and are not used efficiently. Apparently, the time has come to simply change over to large computer centers for collective use.

In the last few years alone science has enriched industry with a number of major new ideas and developments. Powder metallurgy, which is developing before our eyes, and methods of hardening and corrosion resistance coating can be examples of such developments. Laser and plasma technology are invading our life. They open up fundamentally new possibilities for the treatment of materials and the development of efficient technological processes. Methods of nondestructive control of materials and articles are developing rapidly. Significant advances have been made in the development of technology for the production of ultrapure, ultrahard, heat resistant, plastic, composite and other materials.

Often, however, ideas are not rapidly embodied in technological processes and equipment. Such a situation gives rise to a number of major problems in national economic sectors. The concentration of the technological potential is one of the most significant. The establishment of powerful sectorial technological centers with the inclusion in their structure of the corresponding scientific research and technological institutions, as well as experimental bases and production facilities for the manufacture of technological, including nonstandard, equipment could become its efficient form. This would make it possible to solve the problems of development and introduction of energy saving, metal saving, waste-free and other efficient technological processes into the national economy effectively and on a high level. This represents a big field of activity for scientists, production workers, directors of departments and ministries and party organizations in the capital.

The enumerated, as well as many other, problems connected with the development of scientific research and the further acceleration of scientific and technical progress require an improvement in the entire activity of scientific research, planning and design organizations. The decisions of the 26th party congress contain specific instructions for the USSR Academy of Sciences, the USSR State Committee for Science and Technology and ministries on the need to carry out work on the evaluation of the scientific and design base of various sectors and on the development of proposals on the regrouping of scientific forces. This work is already being done.

The commissions of the State Committee for Science and Technology, which included prominent scientists and specialists, checked the activity of 38 scientific organizations of 20 ministries and departments. They made definite advances. At the same time, the work of a number of collectives does not meet the tasks of acceleration of scientific and technical progress. For example, the board of the State Committee for Science and Technology considered the work of the scientific research problem laboratory for automation of production processes at the Moscow Forestry Engineering Institute unsatisfactory. It could not organize the proper communication with the appropriate sector. The USSR Ministry of Higher Educational Institutions was instructed to examine the problem of the advisability of its further existence.

The State Committee for Science and Technology plans and continues to make systematic checks of the efficiency of work of scientific research and design organizations with the participation of scientists at the USSR Academy of Sciences, ministries and departments. We hope for an active participation of the city's party organizations in this matter.

Planning and administration are of great importance in the development of scientific and technical progress. The 26th CPSU Congress determined the need for the further extensive use of program-object methods of planning. Fulfilling the decisions of the congress, the USSR State Committee for Science and Technology, the USSR State Planning Committee, the USSR Academy of Sciences and the USSR State Committee for Construction Affairs approved 160 overall scientific and technical programs for 1981-1985, including 38 object programs. They are characterized by the fact that they are directed toward the solution of scientific and technical problems of an intersectorial nature and encompass all the links of the "science-production" cycle in the area of development of fuel-power and agrarian-industrial complexes, machine building, chemistry, metallurgy, transport, public health, the production of consumer goods and so forth.

A large number of scientific research organizations and industrial enterprises in Moscow participate in the fulfillment of the assignments envisaged by overall scientific and technical programs. Many of them are the head executors of the programs as a whole, or of their individual assignments. Moscow organizations and enterprises will have to fulfill more than one-third of all the assignments included in the programs.

Scientists, engineers, workers and all working people in Moscow, utilizing the accumulated experience and developing the famous labor traditions of the people of Moscow--to be in the first rows of the fighters for scientific and technical progress--are called upon to make and, undoubtedly, will make a worthy contribution to the fulfillment of the tasks set by the party. The development of the initiative that sprang up among the capital's advanced collectives and was approved by the CPSU Central Committee will become a powerful weapon in the fight for an increase in production efficiency and for the saving of national economic resources.

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## ACTIVITY OF EDUCATIONAL-SCIENTIFIC-PRODUCTION ASSOCIATIONS DESCRIBED

Kiev RADYANS'KA UKRAYINA in Ukrainian 8 May 81 p 2

[Article by B. Adamovych and M. Soroka: "Reliable Bridges"]

[Text] Today the Soviet higher educational institution possesses great scientific potential. Almost half of this country's doctors and candidates of sciences are concentrated at higher educational institutions. This is why the task of better and fuller utilization of this potential is so important today. Discussing the possibilities and necessity of improving the system of higher and secondary specialized education, Comrade L. I. Brezhnev emphasized from the speaker's platform at the 26th CPSU Congress: "I have in mind first and foremost quality of teaching and strengthening of the link with production."

History has shown that a reliable path, which leads to great return on the scientific potential of the higher educational institutions, is their close link with enterprises, establishments and organizations, for which they train specialists and perform scientific research work. In recent years a number of interesting and progressive forms of this linkage have developed. Educational-scientific-production associations, which are presently operating at all centers of higher education in this republic, have received perhaps the greatest acknowledgement and dissemination among these forms. What is the "secret" of such popularity? What are the initial results of the activities of these associations? What are their prospects, problems and future development tasks? These and other questions occupied the attention focus of the participants in a round-table discussion held by the department of science and educational institutions of the Central Committee of the Communist Party of the Ukraine, the editorial board of the newspaper RADYANS'KA UKRAYINA, and the UkSSR Ministry of Higher and Secondary Specialized Education.

### Classrooms in Factory Shops

A focus toward practical application is an old tradition of university science. Nevertheless particularly animated activity is being observed today on the "higher educational institution-production" road. This is indicated first and foremost by a steady increase in the economic effect of research which has been and is being conducted by university scientists. The following figure was cited at the round-table discussion: last year alone scientific research at the higher educational institutions of this republic generated savings of 415.6 million rubles to the economy.

The establishment of educational-scientific-production associations (NNVO) is one more convincing example of the strengthening contacts between university science and production.

"In the last five-year plan," stated H. I. Denysenko, rector of Kiev Polytechnic Institute and delegate to the 26th CPSU Congress, "our institute organized five educational-scientific-production associations. A number of prestigious academic and branch institutes, higher educational institutions, and 10 large production associations, such as Arsenal, Tochelektropribor, and the Association imeni S. P. Korolev, functioned as our partners in these undertakings. We had worked in collaboration with them prior to this time, but establishment of the associations has led to appreciably stepped-up contacts. Judge for yourself: thanks to the associations the number of research projects conducted by our higher educational institutions for these 10 enterprises quadrupled in comparison with 1976, while their scope increased sevenfold."

Today there are 116 educational-scientific-production associations within the system of this republic's Ministry of Higher and Secondary Specialized Education. As was emphasized at the round-table discussion by V. I. Kostyuk, UkSSR Deputy Minister of Higher and Secondary Specialized Education, establishment of these associations has promoted accelerated practical application of scientific research results. Here are the results: in the last five-year plan educational-scientific-production associations have brought on-stream 1062 research project results, generating total savings of 110 million rubles. More than 1700 certificates of invention were awarded, and students produced more than 17,000 meaningful course and senior projects.

"We have every reason to state," stated M. F. Kyrkach, rector of Khar'kov Polytechnic Institute, "that establishment of the associations has been very profitable and necessary. Contractual agreements between higher educational institutions and industrial enterprises had existed in the past. Our institute, for example, has maintained relations with a number of Khar'kov plants since the 1930's. But nevertheless today, within the framework of the NNVO, these contacts have become more diversified and clearly defined. We have been joined by academic and branch establishments. Take, for example, the Polikristal Association. In addition to Khar'kov Polytechnic Institute, it includes a Khar'kov plant, the Poltava Synthetic Diamonds and Diamond Tool Plant, the UkSSR Academy of Sciences Institute of Superhard Materials, and a number of branch institutes. This composition provides the capability for combined solution to problems of developing new cutting tools and, most important, efficiently to put obtained results into production."

Having been established on the foundation of existing working relations between university science and production, this new form has comparatively rapidly gained acknowledgement and "right of citizenship." What has fostered this? What forces hold in a common path representatives of different areas of scientific knowledge and production activity, of differing ministerial affiliation? The participants in the round-table discussion responded to these questions with a number of interesting comments, observations, and conclusions.

In their opinion, the motives for active participation by higher educational institutions in these associations lie in the fact that NNVO provide the opportunity to intensify the practical focus of university science, to improve the educational and indoctrination process, to improve the quality of training of specialists, and to improve logistic support of scientific research.

Industrial enterprises are also interested in these associations. They obtain rapid and appreciable assistance in solving scientific and technical problems which arise in production, and in training highly-qualified specialists for their requirements. In particular, this is confirmed by the activities of the Spetsialist Association, the spokesman for which at the round-table discussion was A. S. Havryshko, deputy director of the Institute NDPG [expansion unknown]. He stated that close contacts between NKMZ [expansion unknown] and the Kramatorsk Industrial Institute within the framework of the Spetsialist Association have enabled the enterprise just in this past year to achieve savings of 1.6 million rubles.

As we know, in addition to higher educational institutions and enterprises, academic and branch institutes also are involved in many associations. How do they benefit from this form of collaboration? Chiefly in the fact that it opens up shorter paths for practical adoption of scientific research completed by them. But nevertheless, as is indicated by the results of sociological studies, which were reported to the participants in the round-table discussion by H. M. Dobrov, deputy director of the UkSSR Academy of Sciences Institute of Cybernetics, academic and branch institutes show less interest in the associations than other association members. Various explanations were given in the questionnaires. But in general they boiled down to the statement that NNVO project schedules poorly take into account the specific features and areas of research of academic and branch institute staffs.

Meriting attention in this connection is a suggestion by UkSSR Academy of Sciences Academician Ya. S. Pidstryhach, chairman of the UkSSR Academy of Sciences Western Scientific Center, that the activities of educational-scientific-production associations be channeled toward the tasks being implemented in the 11th Five-Year Plan by the scientific centers. In particular, this would involve more active participation by the NNVO in carrying out combined scientific programs implemented in the interests of a given region or branch.

Other reserve potential for increasing the return of the associations were also stated at the round-table discussion. The necessity of strengthening the experimental-research and production facilities of the members of associations, particularly the higher educational institutions, was emphasized. Unfortunately the ministries and agencies for which the research is conducted do not always show a desire to assist the scientists.

It was also stated in the course of the discussion that a number of important organizational problems pertaining to shaping and managing NNVO activities have not yet been resolved. For example, the councils -- the association management bodies -- contain from 30 to 50 persons each. Such a large quantitative makeup, and in a number of cases territorial delimitation as well, lead to a situation where council meetings are held on an irregular basis and with only partial attendance.
An analysis has shown that the activities of associations frequently are planned without taking into account the basic work plans of the component organizations.

This leads to a scattering of subject matter and diminishes the interests of a number of association partners. The number of branches of departments, scientific research laboratories and instruction centers situated directly at production locations is growing slowly.

Nevertheless, in spite of these shortcomings, the practical return generated from the educational-scientific-production associations is growing. And it is being determined not only by adoption of specific research results into production, not only by a substantial amount of financial savings, but also, what is no less important, by considerable indoctrinational effect. A special discussion of this was held at the round-table session.

## How The Steel Is Tempered

These were unforgettable moments -- metal flowed from the taphole, splashing blinding sparks. It was "their" metal, produced by their own hands, so to speak. Of course to be perfectly accurate, the melt was being directed by experienced blast furnace operators, who were working on their crew. They -- were a group of students at the Dnepropetrovsk Metallurgical Institute.

Institute and plant party committees and Komsomol committees have been initiators of continuous contacts between the future specialists and production people. These committees persistently seek to expand these contacts. An important role in the ideological support of the diversified activities of the Metallurg Educational-Scientific-Production Association is played by the social sciences departments at the institute. Social scientists are doing a great deal of methodological and consultative work with propagandist activists at the Plant imeni G. I. Petrovskiy and the Plant imeni K. Libknekht, which are members of the NNVO. Reporting on this, H. Yu. Samoylenko, party committee secretary at the Dnepropetrovsk Metallurgical Institute, stressed one aspect of the problem -- party monitoring of the activities of the subdivisions of the association.

Unquestionably the role of party committees in the functioning of educational-scientific-production associations should be determining. And especially in coordinating their activities, for an NNVO frequently contains organizations of different ministries and agencies, and the agreement to establish such associations and to conduct joint activities does not yet have the force of a legal document. Everything takes place on a voluntary basis. But even under these conditions it is necessary that the agreement of the NNVO partners be firm, farsighted and carried out conscientiously, with a profound understanding of the importance of the matter.

A. V. Roslavtsev, head of the department of higher educational institutions of t Khar'kovskaya Oblast Committee of the Communist Party of the Ukraine, drew particular attention to the role of party committees in implementation of measures pertaining to studying exchange and dissemination of advanced working know-how of the NNVO. For example, the Khar'kov Party City Committee recently held a scientific-methods seminar on matters pertaining to NNVO activities. Seminar participants included personnel from higher educational institutions, scientific establishments, and specialists from enterprises. The seminar participants drafted recommendations on improving the activities of NNVO, many of which have now been adopted.

Of course the party organizations of NNVO participants should clearly and precisely "plan" feedback between production work forces and higher educational institution staffs. Here in particular one must bear in mind the fact that a considerable portion of the association partners are young people. How are mutual relations formed between students, workers, young scientists, and specialists in the process of their common association, in performing common tasks? In what kind of a moral atmosphere is their mutual collaboration taking place? All these are important aspects of NNVO activities. And naturally these aspects were not ignored by the participants in the round-table discussion. In particular, V. V. Bavin, first secretary of the Donetsk City Komsomol Committee, shared his experience in developing sociopolitical activeness among the participants of educational-scientific-production associations. Here is just one example. The Metallurg-2 NNVO was established in that city, based on the polytechnic institute, the Metallurgical Plant imeni V. I. Lenin, and a scientific research institute. The association totals more than 3200 persons, including 987 scientists and approximately 200 students. A clear understanding of one's role in the association and its tasks, as well as joint, amicable work by young people and experienced specialists have produced fruit. A comprehensive product quality control system, for example, has been developed and adopted. Thanks to this, the percentage share of top-quality product has more than doubled, and metal savings have totaled 2320 tons. This specific program, in addition to savings, also has had an indoctrinational effect -- the young participants in the NNVO can acknowledge with pride that they too have provided a certain impetus to an important cause -- the work force at the Donetsk Metallurgical Plant imeni V. I. Lenin was the initiator of a nationwide competition for the world's finest steel.

## What Stands Behind the Letter

Students at the Zaporozh'ye Machine Building Institute are already accustomed to the fact that lectures, in addition to institute faculty members, are presented by I. M. Dolya, general director of the AvtoVAZ Production Association, chief design engineer V. P. Steshenko, chief engineer S. I. Kravchuk, and many leading specialists from that enterprise. And when attending these lectures, the students frequently comment: "There is nothing about that in the textbooks." And this is not surprising: the latest technological innovations of modern industry and the latest scientific discoveries frequently have not yet reached the pages of the books with which the future specialists are mastering their field of specialization.

Enlistment of highly qualified experts in instructing students has logically become a regular activity of NNVO.

"The first letter of this abbreviated title," stated A. M. Kamorkin, vice-rector of the Zaporozh'ye Machine Building Institute, "signifies that the association has first and foremost an educational function, in the broad meaning of the term. We teach the student through the joint efforts of the institute and production. Instructors also study within the framework of the NNVO, boosting their level of qualifications, going through a period of training at the plant, and plant specialists deepen their theoretical knowledge at the institute, working jointly with our scientists on current problems of machine building."

"I should like to add," interjected V. K. Harachuk, vice-rector of the Odessa Technological Institute of the Refrigeration Industry, "that training of the future specialist is inconceivable without his involvement in scientific work which is directly linked with the daily needs of production. In particular, establishment of branch departments and research laboratories directly at an industrial plant or at scientific establishments which are components of the NNVO may be one effective way to achieve this goal." This was stated not so much as formulation of a question but chiefly for the purpose of sharing already acquired experience. Such experience has been acquired at a number of higher educational institutions, and especially at the Odessa Technological Institute of the Refrigeration Industry. They have set up a special department at a branch scientific research institute, at which, beginning with the third year of study, future engineers specializing in thermal physics can gain a more thorough mastery of their future field.

Scientific research institute scientists guide the students' practical activities and scientific research work. In addition to the professional training of these young people, they perform the functions of mentors and usher their pupils into active participation in the civic affairs of the staff.

The participants in the discussion, especially Yu. M. Hulyamov, party committee secretary at the Dnepropetrovsk Chemical-Technological Institute, and M. G. Makhodkin, corresponding member of the UkSSR Academy of Sciences and dean of the radiophysics faculty at Kiev University, emphasize that in producing a capable specialist it is not enough to furnish him with an enormous store of knowledge and practical skills. It is necessary to teach the future specialist the ability to lead others, to show them an example of creative enthusiasm. Consequently the entire atmosphere of the NNVO and each contact between a student and experienced experts, scientists, leading workers, party and Komsomol officials should become for the future specialist excellent lessons of moral conditioning, political maturity, and the acquisition of organizer skills of the commander of production.

As we see, the educational-scientific-production association, which has become one of the progressive means of linking today's university science with practical activities, at the same time presents its creators with many acute problems. It is therefore not surprising that with unconditional support for the new formula of linking higher educational institutions with industry, the participants in the round-table discussion presented various suggestions for its development and improvement.

H. I. Denysenko, for example, suggested that thought be given to the establishment of a municipal cost effectiveness NNVO, empowered to enlist the services, on the basis of holding two positions simultaneously, of people from industry, scientific research institutes, design offices, and higher educational institutions. H. M. Dobrov proposed the establishment of a republic agency (center) for coordinating the activities of NNVO, enlisting representatives of interested ministries and agencies. In the opinion of Yu. M. Hulyamov, it is extremely necessary, in place of the provisional Statute on NNVO, to promulgate a clearly formulated statute covering its activities, ratified by the USSR State Committee for Science and Technology and the appropriate ministries. The participants' attention was also drawn by a proposal by V. V. Babin to organize socialist competition among the republic's NNVO.

Thus there were many proposals and suggestions, and this is natural, for this is a new innovation which is just beginning to become firmly established. And it is very important today not to sidestep acute questions which arise in the daily activities of the associations, but to seek answers to these questions in a flexible and vigorous manner. Only in this way is it possible to achieve innovative development of this new form of interaction between higher educational institutions and industry. The number of NNVO rose rapidly during the 10th Five-Year Plan. The participants in the round-table discussion believe that it is essential to secure their qualitative growth in the 11th Five-Year Plan.

3024
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## BETTER ORGANIZATION OF DOCTORAL DISSERTATION WORK NEEDED

Moscow IZVESTIYA in Russian 28 Jun 81 p 3

[Article by Professor V. Kirillov-Ugryumov, chairman of the USSR Higher Certification Commission, and Professor L. Sumarokov, director of the International Center for Scientific and Technical Information: "To Augment Science Capital: How to Raise the Value of Dissertations"]

[Text] Further growth in the well-being of the Soviet people is the first priority task of the 11th Five-Year Plan. The party sees the way to accomplish this task as accelerating scientific-technical progress, intensifying the economy on the basis of scientific achievements, and rationally utilizing the scientific-technical potential of the country.

Comrade L. I. Brezhnev, in his report to the 26th CPSU Congress, stressed that "science should be the constant 'disrupter of tranquility,' pointing out what sectors have begun to show stagnation and backwardness and where the current level of knowledge provides an opportunity to move forward more rapidly and more successfully." This is a very responsible assignment, and to accomplish it as well as possible, it is necessary to raise steadily the quality and the level of research, the effectiveness of the work of scientific institutions, and to improve the management of science.

The broad front of scientific research in our country is supported by a large number of factors, including the presence of a large army of scientific workers. In the scientific personnel system, the "capital" consists of scientists with higher qualifications -- the doctors and candidates of sciences, who have made a considerable contribution to the development of national and world science. Although there was a steady rise in requirements for the scientific level of dissertations during the 10th Five-Year Plan, the doctor of sciences degree was awarded to almost 10 thousand persons, and the candidate of sciences degree was awarded to 114 thousand persons.

It is possible to name many dissertations in which innovative solutions to important economic problems are found. Thus, for example, the introduction in industry of the results of the doctoral dissertation of N. Valitov, devoted to the study of catalytic processes, permits the achievement of an economic effect of about 20 million rubles a year. A substantial contribution to raising the effectiveness of metallurgical production is bringing in the application of magnetodynamic pumps for processing and casting alloys, which was researched in the dissertation of V. Polishchuk.

Of course, the significance of dissertation projects is not only valued for its economic effect in rubles. Let us speak of G. Stepanov's dissertation, in which he summarized the method he developed for transplanting fingers and a hand by means of microsurgical techniques. The introduction of this method into practice has put hundreds of people back to work. Or let us take the dissertation of R. Zhuk. On the basis of this author's hypothesis, the anti-swelling preparation "fluorifur" was created; it is now widely applied in clinics in this country and is exported to many countries.

Every week, about 500 dissertations are defended in our country. This figure is tending to increase. To provide maximum effectiveness in utilizing such a flow of scientific works for the reinforcement of human knowldege and for the solution of economic and social problems in the development of the country, it is necessary to take serious measures for improvement in the management of science.

It stands to reason that a majority of completed dissertation projects lie within the realm of urgent problems; however, as analysis shows, too often the degree candidates are working on identical topics. For example, in the field of petrochemistry about 20 percent of the dissertation projects duplicate one another to a significant degree. The same situation also exists in a number of other fields of science. This is evidence of an insufficiently rational use of scientific potential. To a certain extent, the reason lies in the fact that degree candidates and their advisers are not always sufficiently informed about the subject matter of work being done in various research collectives.

How can this shortcoming be eliminated? Here, a whole complex of problems must be solved. But first of all, things must be arranged so that, before approving the dissertation topic, managers of scientific institutions should provide for obtaining from the All-Union Scientific-Technical Information Center (VNTItsentr) of the USSR State Committee for Science and Technology, operative information on what has already been done and what is being done in a given field. This task can be solved more satisfactorily by using the automated information system available in that organization. This system already plays a role, but could play a significantly greater role, as a tool for managing the subject matter of dissertations and as a basis for evaluating the significance of any scientific area and the results of individual dissertations.

Further, in many branches of science there are seminars, the significance of which has extended far beyond the walls of the institutes where they were held. In physics, for example, honored authority is enjoyed by a seminar of the Institute of Physical Problems of the USSR Academy of Sciences, headed by Academician P. Kapitsa; in the theory of automatic control, by a seminar of the Institute of Control Problems, headed by Academician V. Trapeznikov; and in the field of welding, by a seminar headed by Academician B. Paton. We should seek to have the discussion results of such seminars made use of by putting them into the automated information system, after appropriate processing, and to have them become important components of the information base for the certification system. This opens up the possibility for forecasting the preparation of doctoral dissertations in various branches of science.

Broad application of such methods and the utilization of the corresponding information in the activity of the Higher Certification Commission would permit directing work on doctoral dissertations toward more urgent areas of science and technology, after they have received a public evaluation of their significance.

I would like to recall yet another important reserve for improving the mechanism for dissertation certification. It is that, along with the accomplishment of the "immediate" task of evaluating the level of dissertations, the application of automated information systems, like the cases cited above, will permit the Higher Certification Commission to accomplish successfully the very urgent "inverse" task -- the evaluation of the activity of scientists' councils and scientific managers and reviewers.

In the meanwhile, a reviewer who has given erroneous, unfounded praise or unwarranted criticism in his evaluation of a dissertation rarely has any responsibility. This, in the final analysis, is reflected in the level of dissertations. Therefore, the question of increasing the responsibility of reviewers acquires important significance. Expert evaluations of dissertation quality should be stored in the data bank of the automated information system of the Higher Certification Commission. The development of such a system at present is being pushed forward and, in certain of its subsystems, experimental operation has begun. The practical introduction of the system will permit more objective judgment of the network of formal postgraduate training and will increase the personal responsibility of science management for the quality of opinions and reviews and the enlistment in the training of scientific personnel of the largest possible number of doctors of sciences who are working in the field.

An exceptionally important and vital problem is the introduction of dissertation results into practice. To be sure, introduction must be preceded by confidence that a given idea actually has practical significance on a state-wide scale. Giving a reliable answer to this question calls for the objective certification of dissertation projects. The comparison of the scientific and technical results with the highest national and world level also has very important significance in the certification of dissertations. For this also, an appropriate information base in the form of the masses of dissertations, information about them, and other sources is being created in the information centers.

Unfortunately, today the practical checking of the conclusions made in dissertations is frequently left in the hands of the authors themselves. They often procure any kind of references or statements on introduction into production which, verification shows, many times do not correspond to the actual state of things. It appears that the time has come to examine, among the measures for improving the management of scientific-technical progress, a system for effective control over the introduction of dissertation results. Ministries and agencies must become more the organizers for the practical checking of the significance of scientific research in their areas of competence and, consequently, are obliged to control the course of their implementation in production or sphere of education, science, and culture. In this connection, the conclusions of the specialized councils of the USSR Higher Certification Commission, where recommendations are maintained for the most advisable use of dissertation projects, undoubtedly will turn out to be useful. No one can give more qualified advice on the expediency and the ways of introducing dissertation results into practice than specialists in specific fields of science and production.

Of course, the utilization of practical results from dissertation projects makes no sense without precise and specific accounting. The fulfillment of recommendations for the introduction of dissertation results into production must be controlled throughout the full course of implementation.

This kind of accounting and control also must be based on the automated information systems discussed earlier.

In the interests of more rationally using the entire scientific-technical potential, our country is cooperating with other CEMA member countries in the field of information on dissertations that have been defended. This work began in 1973, when the International Center for Scientific and Technical Information began to issue collections of abstracts reflecting the results of dissertation research by specialists of CEMA member countries in all branches of science and technology. The scale of such cooperation is characterized by the following: the total information holdings created in recent years have reached a huge size and consist of about 300 thousand dissertations and accounts of scientific-research projects. It is important to note that an overwhelming number of dissertations done in the USSR have been introduced into the information holdings of CEMA member countries.

Computer processing at the International Center permits the creation of information masses on dissertations with widely varied subject matter and the combining of the dissertation data with data on patents, journals, conference reports, and other publications into a so-called single data base for the defined problem. This is especially important now, when a new task has appeared on the agenda -- the implementation of an effective information supply to complex programs on the most important economic problems, including problems of long-term special-purpose programs of cooperation within the CEMA framework.

Already today, on the basis of the existing information mass on dissertations at the International Center for Scientific and Technical Information, it is possible, with a few hours of computer information processing, for example, to form a complete and precise data base on the economics of atomic electric power stations, on new solar energy technology, and so forth. Such a data base provides the basis for objective evaluation of the status of research on any specific problem and helps to determine better the direction and course of implementing further scientific investigation.

The experience of certifying scientific and scientific-educational personnel in the USSR is causing lively interest among our colleagues in the socialist countries. On the other hand, these countries have very much that is interesting and instructive in the conduct of similar work. Therefore, cooperation in the field of certification is acquiring increasing significance and volume.

In 1980, the Permanent Working Group of the CEMA Committee on Scientific-Technical Cooperation adopted a decision to create a joint expert council for drawing up agreed-upon requirements relating to the main certification questions being raised with respect to dissertations. The same year, in Moscow, an international seminar took place on certification questions, at which there was a wide exchange of opinions and work experience.

At present, within the CEMA framework and with participation by the International Center for Scientific and Technical Information, a number of scientific and organizational measures have been planned that are directed toward the continuation and expansion of effective creative cooperation and the utilization of the positive experience of brother countries in this field. An important role in this cooperation is ascribed to questions of further application of computer equipment and automated information systems for improvement in the system of certifying scientific personnel.

The mechanism of certifying dissertation projects, the role and significance of which are constantly stressed in the practice of socialist construction, continues to develop and be improved as a whole in a number of important areas. To an increasing extent, it is becoming a realistic means for increasing the effectiveness of scientific research and for hastening the utilization of its results in the economy.

It appears that the rational utilization of the mechanism for certifying scientific and scientific-educational personnel, with consideration for its further improvement, will allow science to a still greater degree to fulfill the very function of "constant disrupter of tranquility," about which Comrade L. I. Brezhnev has spoken, in the further development of our economy.

To achieve maximum return from the results of the functioning of the certification system in providing for effective working of the whole mechanism of management is one of the important tasks in the implementation of the decisions of the 26th CPSU Congress.

9645
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## EDITORIAL URGES MORE EMPHASIS ON APPLIED RESEARCH

Minsk ZVYAZDA in Belorussian 3 Apr 81 p 1

[Editorial: "Alliance Between Science and Industry"]

[Text] Fifty million rubles -- this year our economy has already gained this much from application of project results by researchers at the BSSR Academy of Sciences Institute of Nuclear Power Engineering. Hundreds of thousands of rubles of savings have been obtained by research projects pertaining to evaluating various newly developed varieties of fiber flax and hemp. These studies were conducted within the framework of the Len Scientific-Technical Association by scientists at the BSSR Academy of Sciences Institute of Physics, who are working together with the All-Union Scientific Research Institute of the Bast Fiber Industry....

Literally every day brings us new evidence of the excellent results of the close alliance between the creative thinking of scientists and the productive labor of workers at factories and construction sites, kolkhozes and sovkhozes. The integration of science and production is greatly helping this republic's enterprises produce more sophisticated machinery, high-output machine tools and automated production lines, and is helping our farmers boost agricultural crop yields. "The conditions in which the economy will be developing in the 1980's," stated the Central Committee Report to the 26th CPSU Congress, "are making it even more essential to accelerate scientific and technological advance.... The Communist Party proceeds from the position that it is simply impossible to build a new society without science." This assessment of the importance of further development of scientific research places great responsibility on scientists for the results of their efforts, for those research results which they present for practical adoption.

It sometimes happens, however, that scientists lag behind the needs of practical application. It is a fact that some scientific research project results submitted for practical adoption to industrial enterprises are nothing other than new "patches" on old technology, and minor improvement on equipment which for all practical purposes is obsolete. It is also necessary vigorously to oppose tendencies to chase after numbers, at any price, in terms of what is gained from application of research results. It is necessary resolutely to eradicate a practice which still exists in some scientific organizations, of going way out into left field to find economic effect. The tasks of research scientists are quite different -- to offer tested ideas for practical application, ideas which can fundamentally alter the very "countenance" of production. Party organizations of research establishments should play an important role in concentrating the efforts of teams of scientists on solving key problems for the economy. An example of a reasonable combination of basic research and practical application is the Order of the Red Banner of Labor Institute of Physics of the BSSR Academy of Sciences, at which two scientific discoveries have been made in recent years and at which so much effort is devoted to the concrete needs of many branches and sectors of the economy. Elaboration and execution of specific-purpose, comprehensive programs for solving major scientific and technical problems — this is a proven way to increase the up-to-dateness and significance of the creative search by scientists and to increase the yield of research results. Party organizations should focus on promoting competition under the slogan "Every scientific research project to the level of inventions, and every scientific worker an inventor."

Practical application of scientific research results is today a decisive, most acute area of attention. The problem of practical adoption is multifaceted. In this area it is also necessary to increase the personal responsibility of officials of agencies, enterprises and institutes for the manufacture of new products, the employment of advanced technology, and create production incentives for faster movement of innovations on-stream, with development of experimental facilities for testing the ideas of the researchers. One still frequently hears criticism from factories and plants directed toward the scientists for "raw" project results which have not been brought into proper shape, as they say. But sometimes the scientists lack the requisite conditions to do so. It is definitely an acute problem, and measures are being taken in this republic to solve it. For example, plans call for building experimental facilities by 1990, costing many millions of rubles. Today's difficulties, however, should not serve as justification for ineffectiveness. The best solution here — and party organizations should focus scientists on this -- lies in establishing genuinely solid contacts between researchers and the people in industry.

Interesting experience, for example, has been amassed by the BSSR Academy of Sciences. They are working vigorously on drawing up long-range program agreements with branch all-union republic ministries and large production associations. Alongside a number of other advantages, such direct links greatly facilitate and accelerate dissemination of new scientific developments throughout an entire branch.

We could cite many examples of fruitful joint efforts by researchers and practical experts -- involving the BSSR Academy of Sciences, higher educational institutions, and a number of branch institutes. But it was stated at the 29th Congress of the Communist Party of Belorussia that the scientific potential in this republic is not being fully utilized in the interests of increasing the effectiveness of societal production. A close link between science and production should become the standard for every research establishment and for every enterprise. Scientists can — and should -- do a great deal to improve the quality of many machine building industry products -- motor vehicles, tractors, agricultural machinery, and mechanisms. It is necessary to broaden research and achieve more rapid practical implementation of research results in power engineering, mechanization and automation labor, creation of little-waste and waste-free industrial processes, and total utilization of raw materials. Scientists should become actively involved in implementation of the food program. These branches and sectors contain many opportunities for further growth in efficiency of the national economy.

Today, when socialist competition is in full swing for successful fulfillment and overfulfillment of the targets of the 11th Five-Year Plan, it is the honorable task of the work forces of research establishments fully to utilize all reserve potential and to reach new performance levels in broadening and deepening research, more firmly strengthening cooperation between science and production.

3024
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## STRONGER POLICY NEEDED FOR AGRICULTURAL R&D

Moscow SOVETSKAYA ROSSIYA in Russian 28 Apr 81 p 1

[Editorial: "Science for Agriculture"]

[Text] Whatever the question being decided today in farm areas — whether production should be mechanized, whether there is growth in harvests and cattle productivity, construction, or land development, or whether social and welfare inquiries are satisfied — it is certain that science is providing recommendations in answer. Now, hardly anyone needs to be convinced that further improvement in agriculture and increase in its productivity are simply inconceivable without science. The decisions of the 26th CPSU Congress require increasing attention both to utilizing the achievements of scientists and to developing science itself. Much is being done in this direction. Agrarian scientists have provided tens of new varieties of agricultural crops, have developed new technology for land cultivation and livestock breeding and complex measures for protecting the soil from erosion and for effective utilization of reclaimed and hilly lands, and they have made a large contribution to solving social problems. All this has permitted an increase in products, although the number of rural workers has declined.

"Today, looking forward for five and for ten years," said Comrade L. I. Brezhnev at the 26th CPSU Congress, "we cannot forget that it is during these years that the economic structure will be put together with which the country will enter the 21st Century. It must embody the basic characteristics and ideals of the new society, be in the vanguard of progress, and personify the integration of science and production, the inseparable union of creative thought and creative labor."

These characteristics already can be seen. But it is necessary to achieve even closer and, most important, more fruitful union between science and production. In the 1980's it has been planned to complete a transition of all economic sectors to a primarily intensive mode of development. The results of production must grow faster than the expenditures for them. In agriculture, this task is being accomplished poorly.

It is necessary to concentrate the efforts and the means of agrarian science on key areas. One of these is productive utilization of land. Completed research projects which have developed recommendations for increasing the fertility of fields still are not providing the needed results. In any zone, let us say, a nonchernozem zone, there are kolkhozes and sovkhozes that harvest 30 to 40 centners of grain per hectare, but there are many that achieve half as much. Of course much depends here on by whom and how the land is managed. However, one cannot belittle the role of science in this. It must be, according to Comrade L. I. Brezhnev's way of expressing it, "the disrupter of tranquility."

Take grain farming, for example. This is the basis for the whole food complex of the country. Much has been done by scientists, so that Russian kolkhozes and sovkhozes now harvest 134 to 136 million tons of grain per year. Agricultural workers highly value this contribution. However, there is still instability in this sector. In the last ten years, gross grain yields fluctuated from year to year in the Russian Federation from 77 to 137 million tons. This has had an extremely negative effect on livestock breeding, since, in years of bad harvest, kolkhozes and sovkhozes cut back the number of cattle and lowered the productivity. Instead of moving forward, it took years to recover what was lost.

Agrarian science explains why this happens. But just to explain the reasons is not very much; it is necessary to have an active influence on productive activity and knowledge of how to avoid undesirable phenomena that arise from difficult weather conditions and how to reduce their negative consequences. It is here that agrarian scientists are falling short.

This also pertains to problems of feed production and livestock breeding. Needed development of research has not been achieved in the specialization and concentration of production, interfarm cooperation, formation of an agroindustrial complex, and improvement in organizational forms of management. Life suggests that it is already necessary to have model farms of the future. Searches are being conducted, and there are concrete results which should be examined and studied. One of these was recently discussed in SOVETSKAYA ROSSIYA by the director of the Novosibirsk sovkhoz "Berdskiy" and Hero of Socialist Labor, Comrade I. I. Leunov. After adopting a new management structure, this sovkhoz became essentially an agricultural combine. A high effectiveness was achieved in all areas. Perhaps this is the right model for a future farm. It would be useful for both scientists and the RSFSR Ministry of Agriculture to investigate carefully the results of the experiment. Any innovation requires serious interestedness and thoughtful work . Only then can it be put to use.

The weakest link in combining science and production today remains the introduction of discoveries and achievements. Many valuable developments and recommendations are not applied for a long time. Certain new varieties and hybrids of grain crops are being advanced slowly on the experimental plots of kolkhozes and sovkhozes. In a number of oblasts, this takes up to 10 years. In arid steppe rayons, insufficiently broad use is made of the soil protection system of cultivation and the autonomous republics of the Northern Caucasus poorly follow scientists' recommendations that have been developed for irrigation farming. For this reason, at many kolkhozes and sovkhozes, the yield from irrigated lands differs little from that of nonirrigated lands. But meanwhile, there are special organizations that are obligated to engage in innovation -- cost accounting centers, scientific-production laboratories that have thousands of associates on their staffs and, in oblast agricultural administrations there are groups for innovation. Experimental-demonstration base farms have been organized, where high priority is given to the application of scientific achievements. The results of their activity, however, give no cause for rejoicing.

Here the aid of party organizations is necessary to raise the level of institute and laboratory responsibility for the effectiveness of recommendations. Lack of discipline and control engenders inertia and passivity in some scientific workers.

Kolkhozes and sovkhozes have complained for years to the collective of the Scientific-Research Institute for Potato Farming: selection work on varieties that possess good taste qualities, resistance to disease and insects, are suitable for mechanized harvesting, and capable of long storage has been poor. But the situation is slowly changing for the better.

The time has come to put the introduction of scientific achievements on a good planning basis and to create a solid material basis for this. This requires, possibly, breaking outdated habits and indicators. And it must be approached boldly. It cannot be managed without active assistance from party committees. They have been called upon to give serious attention to the organization and work style of scientific institutions and the level and quality of their research, and to create an atmosphere of strict standards and responsibility for the effectiveness of scientific developments.

A scientist is always searching for what is new. This is his need. And if someone's need becomes dulled from complacency or because the unfamiliar path promises undesirable anxiety for settled authority, then caution arises and, with this caution, stagnation. Individual scientists think there is no reason to hurry developments for improving quality of pedigreed milk cows. Supposedly there is no necessity. Such a revelation, heard at a recent prestigious meeting surprised many. Productivity as a whole of the milk herd of the oblasts, krays, and autonomous republics of Russia remains low. Two or two and a half tons of milk per cow per year -- such a milk yield can satisfy no one. Communists are called upon to speak decisively and act uncompromisingly against stagnation, complacency, and uncritical evaluation of what is achieved.

Science looks ahead a long way. Today, agrarian scientists talk not about providing machines of one kind or another to the countryside, they are concerned with creating systems of machines and providing complex technology. They conduct research on the utilization of atomic, solar, and geothermal energy in agriculture. This puts special responsibility on farmer-managers, specialists at all levels, mechanizers, and on everyone engaged in agricultural production. They must be ready for the changes being dictated by scientific-technical progress. Unfortunately, in many rayons, kolkhozes, and sovkhozes, the degree of such readiness is low. This is exactly the reason that in Yaroslavskaya and Kalininskaya Oblasts and in the Udmurtskaya ASSR, the recommendations of science and its long-range trends are regarded without proper attention and necessary motivation and, on some farms, the attitude is irresponsible.

In helping to overcome these shortcomings, local party committees are also showing concern about the organization of science itself. The regional departments of the All-Union Academy of Agricultural Sciences imeni Lenin that have been created still do not solve problems in a complex manner and scientific efforts are allowed to be scattered. All this requires increasing the role of the Agricultural Academy, providing a unified scientific-technical policy, the elimination of parallelism, and effective joint activities both by those of economic sectors and of regions. The time has come that coordination of activities becomes cooperation of scientific efforts.

Under these conditions, work with personnel must have a more purposeful and long-range character. In a number of institutes the flow of young people to agricultural science has decreased. The reasons for this should be examined seriously. Improvement in the training and certification of personnel and the correct combining of the energy of young scientists with the rich experience of authoratative, well-known scientists constitute an urgent necessity of the times.

Agrarian science in these stages has made a worthy contribution to the development of agriculture. The large size of the tasks of the 11th Five-Year Plan are pushing it to new heights. Close teamwork with practice will help occupy these heights and implement successfully the decisions of the 26th CPSU Congress.

9645
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## INTERBRANCH ORGANIZATIONS NEEDED TO OVERCOME INSTITUTIONAL BARRIERS

Riga SOVETSKAYA LATVIYA in Russian 8 May 81 p 2

[Excerpt from article by Yu. Mikhaylov, director of the Institute of Physics of the Latvian Academy of Sciences: "How Can the Barriers Be Overcome?; The Union of Science and Labor"]

[Excerpt] With the problem of relations between science and production, the "ice is broken," as they say. A new form of organization has arisen -- scientific-production associations. They contain all the elements necessary for rapidly moving ideas from development to industrial assimilation. In science and in economics, projects are being worked on according to special-purpose complex programs. Nevertheless, the path from science to practice is still not much of a thoroughfare.

During the years of the 10th Five-Year Plan, 367 large developments by the Latvian SSR Academy of Sciences were introduced into the economy. Some of these have already been told about here. But the problem of introduction is far from optimum solution. Things go better in those institutes that have their own experimental enterprises and design bureaus. Here they can develop devices, machines, or technology to the point where they can be adopted easily by industrial enterprises. That is why the decisions by the congress speak of the necessity for strengthening the supply and equipment base of scientific-research, exploratory-planning, and design organizations and higher educational institutions. Unfortunately, the plans for capital construction under the "science" heading in our republic are still not fully fulfilled. But, in its turn, science, too, is in debt to the republic. Institutes of the physical and engineering department hand over to enterprises located within the republic less than 20 percent of what is handed over in other republics of the Soviet Union.

The reason lies not in the absence of a desire on the part of the institutes to establish local contacts. The examples introduced demonstrate that. Although the institutes maintain relations with individual enterprises, more rarely with ministries, the number of such economic areas is not great. Our republic is a complex region. Here, many all-union and republic economic sectors operate, and difficulties arise in overcoming inter-sector barriers both in implementing complex inter-sector programs and in a simpler matter -- the introduction of more or less uniform manufactured items and instruments at similar enterprises of different sectors.

Here is a characteristic fact. "EMAGO" is a trade mark registered by the Institute of Physics and stands for a whole complex of electromagnetic noncontact means for identification, orientation, control, sorting, and cassetting of parts. The method is especially hopeful in electrical engineering, radio engineering, instrument-making sectors. The growing variety and complexity of the items being produced in these sectors is leading to the appearance of a large number of types of components and assemblies, the processing and assembly automation for which is usually difficult or impossible, with many operations done by hand as before.

"EMAGO" methods and devices permit in many instances, the solution of these complexities. High productivity, universality, and rapid rearrangement of systems for a new range of items are being successfully achieved. Now "EMAGO" is operating at many enterprises of the country and negotiations for the sale of licenses are being conducted. Finally, interagency commissions of the ministries recommended series production. But there is still no series production, although many years have passed.

Why is this? Industry was used to large batches of uniform items; then, production was effective and economical. But a ministry did not need so many. In order that hundreds of systems would turn into thousands, their manufacture had to be done in one place for various sectors, but the same agency barriers would interfere.

The way out of this situation, in my opinion, lies in the creation of interagency firms and special design bureaus with experimental facilities. Such organizations already exist on a cost-accounting basis at higher educational institutions and republic academies of sciences. This experience must be studied for the elucidation of the best conditions for its application.

Since a universal mechanism for successfully overcoming interagency barriers still has not been developed, it is apparent that it would be advisable to utilize the experience of Leningrad, Novosibirsk, and L'vov party organizations.

In L'vov, for example, they have started forming interagency special-purpose scientific-production associations and, on this basis, complexes (machine-building, instrument-making, geological-geophysical, and others). Two principles were placed as the basis of organizing such associations. The first was the preservation of legal and administrative independence of organizations that established nongovernmental cooperation on a mutually advantageous basis. The second was the coordinated use of material and personnel resources with continuous interagency planning.

The management of interagency special-purpose complexes in a region is entrusted to directors of institutes and enterprises and is accomplished in close interaction with managers of corresponding departments of the party obkom. It is the interested participation by party workers that makes possible the strengthening of coordination among economic sectors. Party management permits, on the one hand, the establishment of business-like mutual relations with ministries and agencies and, on the other hand, provision for regional interests. Such an approach was successful in consolidating subject-matter, reducing time-lengths for introducing scientific research, and establishing trust between various organizations participating in the work. In a short time, over 200 scientific projects were introduced with a total economic effect of 130 million rubles.

For basic improvement in innovation, it is desirable in our republic also to unite into a single whole the efforts of our scientists, producers, and party bodies.

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## ORGANIZATION AND PROBLEMS OF COMPREHENSIVE INTERBRANCH PROGRAMS DISCUSSED

Moscow PRAVDA in Russian 14 May 81 p 3

[Interview of I. G. Gverdtsiteli, chairman of the GSSR State Committee for Science and Technology and vice-president of the GSSR Academy of Sciences, by journalist G. Bel'skaya: "A Living, Creative Operation: Complex Programs"]

> [Text] In the past five-year plan, for the first time in our country, there was a broad use of solving large scientific-technical problems on the basis of complex programs involving more than one economic sector. Sometimes, many organizations of various ministries and agencies participated in the fulfillment of these programs. And, when effective interaction was achieved among them, the results achieved turned out to be significant. Special-purpose program planning is a live, creative operation, and its development was given a large amount of attention at the 26th CPSU Congress.
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> This method has also received notable implementation in Georgia. I. G. Gverdtsiteli, chairman of the Georgian SSR State Committee for Science and Technology and a vice-president of the republic Academy of Sciences, in a conversation with the journalist G. Bel'skaya, analyzes the experience that has been accumulated and notes the shortcomings and difficulties that have to be overcome for further improvement in the practice of working by complex and special-purpose programs.

[Question] How is the complex special-purpose method for planning developing in the republic today?

[Answer] We are now fulfilling over twenty scientific-technical programs, which cover a wide range of research and development projects in industry, agriculture, and health, and include new machines and equipment, technological processes, and materials. In all the programs, coordination councils have been created; the managers have been confirmed; head ministries, agencies, and organizations have been determined; and the supply, equipment, and financial resources necessary for fulfillment have been distributed. These programs form a basic part of the five-year plan for the development of science and technology in the republic.

[Question] Could you characterize some of these in more detail?

[Answer] As is well known, Georgia grows a significant portion of the southern and subtropical crops that are cultivated in the country. The demand for them keeps growing, but the possibilities of the republic are limited. There has been a question about increasing the effectiveness of cultivation in the lands of the Kolkhida Depression -- a basic reserve for increasing the production of southern crops. But for this, it was necessary to develop and scientifically substantiate a system for draining marsh lands, a more rational plan for locating plantations, agrotechnical methods for effective cultivation of hard soils, protection of plantings from frost, and so forth. As a result, the "Kolkhida" complex special-purpose program was formulated, the implementation of which occupies seventeen scientific-research organizations.

The inter-sector character of a scientific-technical problem was clearly expressed in the program for the complex processing of the copper ores of Madneuli and the manganese ores of Chiatura. Institutes of the Georgian Academy of Sciences developed a technological process for producing from these ores dioxides of manganese for the electrical engineering industry and ultradispersion copper powder of high purity with uniform shape and size of particles. For the development of technology under production conditions at the Madneuli Mining-Enrichment Combine, a semi-industrial plant has been equipped. As the result of lengthy experiments, a technology was successfully created that permits extraction from the ore not only of copper and manganese, but also iron and rare metals. Thus, the new technological process responds to the interests of the chemical industry and to those of nonferrous and ferrous metallurgy. There are grounds for thinking that completely developed industrial technology will be completely without waste products. A guarantee of this is the purposeful close cooperation between scientific workers and production workers.

The automated control system "RASU-Gruziya" [Republic Automated Control System -- Georgia] being developed in the republic continues to be improved. It has been called upon to integrate the systems of economic sectors and to distribute to directive bodies the necessary information and variants of solutions for optimum economic management of the republic. An affiliate of the All-Union Scientific-Research Institute for Problems of Organization and Management was created especially for the development and exploitation of "RASU-Gruziya", and it has taken on the scientific management of this complex special-purpose program. At the present time, individual fragments of it are being developed, which have great independent scientific and practical meaning.

In the past year, we were successful in fully implementing one such fragment as an example -- "ASU-Rtveli" -- in controlling grape harvest in Kakhetiya. Originally, the system was tested in a typical Georgian agricultural rayon -- Telavskiy -- in 1978. After that, as the automated control system for the grape harvest process was successfully implemented within the boundaries of that rayon, the experience was disseminated throughout Kakhetiya. According to preliminary calculations, the economic effect of its application in 1980 exceeded three million rubles.

[Question] What are the general principles of program control?

[Answer] Concrete tasks are assigned and delimited between the implementing partners -- scientific-research organizations and enterprises. A specialized scientific-technical council has been created for each program at its head institute. Every year, the programs are revised, are checked for progress in implementation, and are improved constantly. Thus, complex special-purpose planning is a really dynamic, flexible, and constantly improving system of control. "It must be generally noted," said Comrade L. I. Brezhnev at the 26th CPSU Congress, "that improvement in the organizational structure of control does not tolerate sluggishness." Special-purpose program planning, in my view, fully responds to this requirement.

[Question] There are probably some difficulties in the course of formulating and implementing programs . . .

[Answer] Of course, everything does not go smoothly everywhere. The greatest difficulty is managing programs according to the plan. In itself, planning scientific developments is not an easy matter; everything here cannot be arranged strictly on a chart, especially when you have to travel so-called unbeaten paths. That is why the coordination of work by co-performers requires an efficient information system. But even this only helps partly. There are continual difficulties in providing material resources and equipment to the program task performers.

Considering the experience of the past five-year plan, one can make this conclusion: We have begun to plan the solution of large scientific-technical problems and to unite the efforts of scientific-research organizations and industrial enterprises. But we still have not fully mastered the mechanism of controlling these complexes and we do not always know how to restructure things on the right course. Complex special-purpose programs are self-developing systems insofar as their fulfillment constantly poses new tasks. And it is necessary to react effectively and innovatively to this. Such was for us the lesson of the 10th Five-Year Plan, but this is also the task for the 11th Five-Year Plan. Here, there will have to be improvement in the coordination of efforts by Gosplan, Gossnab, the GSSR State Committee for Science and Technology, and the republic Academy of Sciences.

Recently, in composing the plan for the development of science and technology, a special section was included: "The first introduction of a scientific development into practice." This has special interest as the precursor of the serial assimilation of new products and is called upon to ease the difficult procedure for putting scientific achievements into practice.

[Question] The 26th CPSU Congress gave a large amount of attention to the acceleration of scientific-technical progress, the introduction of scientific and technical achievements into the economy. What are the optimum ways for achieving these?

[Answer] Today, unfortunately, not all our scientific-research organizations possess opportunities to carry scientific research forward to the stage where its results can invoke the direct interest of industry. On the other hand, industrial enterprises do not always have units that are able to implement in production the technology that has been developed at scientific-research institutes. To overcome this difficulty, we also practice creating semi-industrial facilities by local efforts of scientific and production organizations. This is an effective form of integration of science and production, as both scientists and producers speak in full measure as the creators of developed technology and corresponding equipment.

Another aspect is the maturing of independent demand in the economic mechanism, which would raise the level of interest by industry in the creation and introduction of new technology. Unfortunately, introduction of innovations often lowers the economic indicators of an enterprise. The CPSU Central Committee and USSR Council of Ministers decree On the Improvement of Planning and Strengthening the Influence of the Economic Mechanism on Increasing the Effectiveness of Production and Quality of Work" provides a stimulus for innovation activity.

And still another important problem: We talk much about aid by science to production. However, it should not be forgotten that science needs help from industry, because it relies on the latest achievements of technology itself, especially instrument-making. Therefore, science is to production as production is to science; they are inseparable links in one chain. And both these links have a primary role in the intensification and further increase in the effectiveness of socialist production. In short, as Comrade L. I. Brezhnev noted at the 26th CPSU Congress, "Close integration of science and production is the urgent requirement of the present era."

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## UNWIELDY PLANNING MECHANISM HINDERS S&T DEVELOPMENT

Moscow PRAVDA in Russian 10 Jun 81 p 3

[Article by N. Stankevich, director, and M. Mazin, sector chief, of the All-Union Scientific Research Institute for Installation and Special Construction Work: "Paper Merry-Go-Round: On Complex Programs"]

[Text] The increase in effectiveness of the economy to a significant degree is determined by how fast scientific-technical innovation can be created and carried forward to massive introduction. Its development and assimilation require a complex approach throughout all steps--from scientific research to industrial prototypes and farther--to serial production.

Neither a single scientific-production association nor even a single very large ministry is often in a position to cover all these steps with the efforts and means available to it. Various organizations of different ministries and agencies most often fulfill individual stages of development, especially in the case of large developments. Success here depends on the interaction of tens and, at times, hundreds of organizations and enterprises whose interests do not always coincide. As the result, innovations are often delayed, sometimes for a long time. The answer is to supplement functional-agency management with special-purpose programming, which received much attention in the Basic Directions for the Development of the Country that were adopted by the 26th CPSU Congress.

They make serious demands on program content as well as on preparation and fulfillment. They also were placed at the base of developing scientific-technical programs in the USSR Ministry of Installation and Special Construction Work. Analysis has shown that no important problem in the development of an economic sector can be solved in isolation from others. Thus, raising the level of mechanization and reducing the proportion of manual labor are related to increasing the expenditure of fuel and energy, and economy of metal often causes increases in the labor content of manufactured metallic designs. Isolated development of programs for these problems can lead to serious miscalculations. Therefore, sector programs have not been developed separately but as an interrelated "package."

A large part of them pertains to such ministerial ends as the introduction of new technology for fulfilling types of installation and special projects. Each of the corresponding documents coversall stages of development and assimilation not only of concrete technology, but also the creation and introduction of new materials, the standardization of processes and products, and capital construction. For the conduct of a unified technical policy, the coordination of projects, and the convenience of approval, these types of tasks are carried in separate functional programs.

For their coordination one with another and with the five-year plan, technical-economic indicators have been unified. This has made it possible to bring out the need for basic types of resources and sector and economy effectiveness not only of an individual program but also of their sector "package" as a whole.

The aspiration of using programs as a basis for controlling the development of an economic sector, however, has clashed with the disorder of the planning system.

Broad application of programs supposes careful elaboration and maximum simplicity in their contents. But this has not been successfully achieved. USSR Gosplan has complicated their development by deciding to distribute nine forms for economic programs for sectors. Just the listing of the basic tasks of our ministry's program takes 160 pages. Filling in these forms turns programs into an unwieldy document that is difficult to control. Meanwhile, all this planning documentation could be fully carried on three forms, characterizing the basic program tasks, stages for their fulfillment, and requirements for resources.

The USSR State Committee for Standards and other state committees have introduced their own forms of documentation. Not having unified documentation for programs, their developers must transcribe the same task to the various planning documents. Still worse, in this connection, the effect being achieved from fulfilling the tasks is repeatedly summarized and, in the end, it does not provide complex solutions to problems. Documentation for programs must be simplified and unified.

Serious difficulty is caused by approval of programs that are sent to 40 ministries and agencies. Some ministries, having been referred to operative state standards, have declared that they have to submit documentation that corresponds to such standards rather than to Gosplan forms. Others require preliminary approval from their lower organizations although, by Gosplan instructions, programs are supposed to be agreed to my ministries and agencies themselves. A third group simply refuses to participate in fulfilling "somebody else's" program because of overwork. The five-year plan has begun, but it remains unclear how to achieve the good graces of a number of ministries and agencies whose participation is essential for implementing the programs of the Ministry of Installation and Special Construction Work.

It is no less complicated to put together ministerial plans based on programs. Forms for five-year and yearly plans are not uniform with forms for programs. Still worse, a number of indicators in these documents just do not "jibe."

Many complications are caused by the absence of interrelations between programs and commission-orders; they duplicate one another in content, but they have to be agreed upon and confirmed separately. It would be useful to draw-up any tasks for the development and introduction of innovations in program form and include them among the commission-orders.

Thus, even at the stage of developing programs and drafting plans on their basis, obstacles arise which lower the effectiveness of special-purpose program management.

Many difficulties relate to organization of work on program fulfillment. Each of them should have a management body, allotted sufficient rights, financial, and other resources, and also incentive funds. It is also important to provide qualified assistance to the implementation of programs, especially at the stages of development and study of innovations, their experimental and experimental-production trials, and their introduction into production. This matter can be assigned to head institutes in economic sectors, after their material base has been strengthened. It is evident that USSR Gosplan and the USSR State Committee for Science and Technology should examine these problems and adopt appropriate solutions.

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# NEW SYSTEM OF PLANNING INDICATORS IMPROVES EFFECTIVENESS OF LENINGRAD MECHANICAL INSTITUTE

Leningrad LENINGRADSKAYA PRAVDA in Russian 10 Jul 81 p 2

[Article by G. Luk'yanov, prorector for scientific work, Leningrad Mechanical Institute]

[Text] Science has ceased being a vocation for gifted individuals. Today it is the field of activity of tremendous collectives. They possess the desire for research and love of knowledge, but at the same time other regularities are in operation. The role of competition and moral and material stimuli is sharply growing, and their influence on time schedules and quality of work is becoming decisive.

Large funds are being invested in science today. The search of ways of increasing the return from each expended ruble is especially important. As emphasized at the 26th CPSU Congress by L.I. Brezhnev, it is essential "to improve the organization of the whole system of scientific research. This system should be significantly more flexible and mobile, not tolerating nonproductive laboratories and institutes."

What is needed to boost the effectiveness of the researcher's work is to learn how to objectively evaluate importance of results and to be able to compare them with each other. Three years ago, our institute was given the task: to develop a system of controlling the effectiveness of scientific research. In creating it, we depended on the experience accumulated at a number of the country's leading VUZ's and on the study of methods of assessing the importance of scientific work performed at our institute under the leadership of Professor I.M. Tkalin.

The basis of the system is to be found in a "set" of planning indicators for the work of departments. Annual targets for a number of most important parameters as fulfillment of thematic plan, introduction of the scientific activity of students for publications and others are set for them. The work of creative collectives is many-sided, for which reason the number of evaluative parameters can change from year to year: the system, not dogma, has to be developed.

Introduction of the system has made planning easier. The work of each scientific subunit and its share can now be seen much better than formerly. It was found that good indicators of the institute were attained far from the individual efforts of departments. Let us say that eight of them submitted 60 percent of all announcements of inventions instead of the 40 according to plan. The other showed results that were clearly below their possibilities. The demands on the laggers were increased, and here is the result: last year two-thirds began to work significantly more actively.

At the same time, a check was carried out at the institute of the system of controlling the effectiveness of the scientific-research work of the department. It helps evaluate the quality of the work of thematic groups and colleagues and adopt a decision on moral and material incentives. All processing of information is done with the help of a computer. The system has made it possible to disclose more fully the scientific reserves of the department and to redistribute cadres and resources with a better method.

Thus, the system of controlling the effectiveness of scientific-research work in general outline came into being. There is planned in the immediate future machine processing of information.

I shall give some figures to illustrate the effectiveness of the system. On the whole, during the past five-year plan, with a constant number of workers the volume of scientific research at the institute, compared to the Ninth Five-Year Plan, grew 1.3-fold and the economic effect increased 1.7-fold. There were considerable more major developments of importance to production: the average "size" of a scientific theme doubled. But the last two years of the five-year plan--a time in which the system was in operation--were markedly outstanding in their results even against such a background. For example, the number of announcements of inventions submitted in 1979 and 1980 was of the same number as that of announcements for the preceding three years. The growth of the work efficiency of the institute was due to a significant degree to the "hauling up" of lagging collectives to the level of the leaders.

An important quality of the system is its close connection with socialist competition at the institute. The results "put out" by the automated control system served as the chief criterion in the distribution of class places according to the results of the competion and moral and material incentives. They lend themselves to wide-scale publicity. It is at times difficult to sometimes be able to name the best in the scientific collective. The rating system that we formerly used for determining of winners sometimes gave rise to significant errors: say a laboratory took first place; at the same time it was clear that its rating came from second-rate directions. Today that would be simply impossible.

Without a doubt our developments are far from perfection. The system is in need of a serious "adjustment," and of a closer connection to the practice of certification and competitions. The assessment of the importance of scientific work has been so far of an approximate character. But even in its initial stage, the system is producing good results, and it can develop continuously.

From the very start, the development and introduction of a system of control over the effectiveness of scientific work took place at the institute with the active support of the party organization. The tasks of the collectives and the results of fulfillment of plan indicators were always under the observation of the institute's council, party and local committees, party and trade-union bureaus of the faculties.

The system of control of effectiveness of scientific research has done much for the institute's collective. At the same time, it has graphically shown that the organization of VUZ science contains big, so far not utilized, resources for growth of the tempo and quality of research. The chief obstacle here is the present procedure of financing work. The economic contract provides for issue to the VUZ of certain funds which must be used in the course of a certain time, after which the client is to be presented with a ready result. At the same time, measures are not provided for rewarding those collectives that were able to achieve results more quickly and less expensively. Moreover, such collectives subsequently find themselves in a more difficult financial position-- their wage fund is "cut," and they are "punished" by reduced bonuses. Consequently improvement of the system of financing VUZ science is a most important task. We believe that Leningrad's council of rectors would find it worthwhile to come out with suitable proposals to the Ministry of Higher and Specialized Secondary Education and USSR Gosplan.

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## BSSR GOSPLAN OFFICIAL ON IMPROVEMENTS IN S&T PLANNING

Minsk ZVYAZDA in Belorussian 16 Apr 81 p 2

[Interview with Viktor Aleksandrovich Gvozdev, chairman of BSSR Gosplan, first deputy chairman of BSSR Council of Ministers, by ZVYAZDA reporter Ul Narkevich: "Effectiveness of Scientific Search"]

> [Text] A scientific and technical conference entitled "Improving Planning of the Development of Science and Technology at Various Levels in Light of the Resolutions of the 26th CPSU Congress" convenes today in Minsk. It is being sponsored by the USSR State Committee for Science and Technology, the BSSR Academy of Sciences, and BSSR Gosplan. The editors of the newspaper ZVYAZDA asked Viktor Aleksandrovich Gvozdev, first deputy chairman of the BSSR Council of Ministers and chairman of BSSR Gosplan, to say something about the most important questions which will be discussed by prominent Soviet scientists and on the problems which must be resolved in order to ensure acceleration of scientific and technological advance.

[Question] Please tell us, Viktor Aleksandrovich, what recent new developments have taken place in planning scientific research?

[Answer] Scientifically substantiated planning is a proven and reliable instrument of practical implementation of the economic and social policy of the Communist Party of the Soviet Union. Vladimir Il'ich Lenin saw in planning a means of control and management of the national economy. The economic and social policy elaborated at the 26th CPSU Congress is embodied in national economic plans, which constitute an organizing force in accomplishing the tasks elaborated by the party and in our homeland's movement along the road to communism. The conditions in which the national economy will be developing in the 1980's make it even more essential to accelerate scientific and technological advance. "The Communist Party proceeds from the position that building a new society without science is simply impossible," stated Comrade L. I. Brezhnev in the Central Committee Report to the 26th CPSU Congress. Acceleration of scientific and technological progress is viewed in the congress proceedings as a mandatory component part of the principal task of the 11th Five-Year Plan, while development of science is the basic foundation of scientific and technological advance. As we know, the CPSU Central Committee and USSR Council of Ministers specified in the decree entitled "On Improving Planning and Strengthening

Effectiveness of the Economic Mechanism on Improving Efficiency of Production and Quality of Work" a system of measures to improve the mechanism of management of scientific and technological advance. Particular attention is focused on planning and management in the area of science and technology, with efforts to be concentrated in the principal areas of scientific and technological advance, with accelerated adoption into the nation's economy of the latest scientific and technological advances, and with the efforts of scientific and design organizations and enterprises focused on end results. Much is being done in our republic to implement the program of accelerating scientific and technological progress elaborated by the party. The Central Committee of the Communist Party of Belorussia and the BSSR Council of Ministers issued a decree in December 1976 entitled "On Measures for Further Improving Planning of Scientific Research Activities and Accelerating Adoption of Research Results Into Production." In the course of implementation of this decree, a new approach to organization of scientific research and passing on research results to the nation's economy was elaborated and has been successfully implemented in this republic. In the past planning of scientific research was carried out by the researchers themselves, which led to insufficient consideration of the needs of production, while the economic order is now becoming the basis of planning. Ministries and agencies submit to BSSR Gosplan order-proposals, which specify research topics, present the technical and economic substantiation of the expediency of their elaboration, and specify the requested executing agency. Following discussion and coordination with the BSSR Academy of Sciences and MinVNU [Ministry of Higher Educational Institutions], as well as with branch scientific establishments, the most urgent proposals become plan topics; they are provided with financial and material resources. BSSR Gosplan is empowered to include in the national economic plan promising scientific research topics and areas, and is also empowered to halt research projects which are not of current significance. This practice of planning and financing the most important scientific research, experimental-design and engineering projects is producing good results. In the 10th Five-Year Plan scientists of the Belorussian Academy of Sciences were awarded two Lenin Prizes and two USSR State Prizes for great achievements in the area of science and technology; three major discoveries were registered. The innovativeness of scientific and technological development projects carried out at academic establishments has been confirmed by the award of more than 4,300 certificates of invention. Savings from the direct practical adoption of the results of projects of scientific establishments of the BSSR Academy of Sciences, this republic's ministries and agencies totaled 890 million rubles in the last five-year plan. The people of our academy, as well as of other scientific and scientific research establishments, intend to continue building upon achieved successes in the 11th Five-Year Plan. During this period plans call for execution of 50 scientific and technical programs in Belorussia, which will encompass an entire aggregate of scientific research projects, from initial idea to practical adoption.

[Question] In your opinion, what reserve potential exists for increasing the current significance and importance of research being conducted by the scientists of this republic, and to what areas of research activity should they devote particular attention?

[Answer] In spite of the considerable success which has been achieved by this republic's scientific establishments together with planning agencies in the area of planning scientific research activities, their activities are not yet fully in conformity with the requirements elaborated at the 25th CPSU Congress and 29th Congress of the CPB. Selection of the most effective paths of scientific and technical search is acquiring great importance today. First of all, science and technology development plans should provide for purposeful and effective concentration of the manpower and resources of science on accomplishment of a strategic task of economic development -- improvement in the efficiency of the nation's economy. We expect of science the elaboration of fundamentally new scientific-technical, organizational, design and engineering solutions, which will make it possible to revolutionize industry, to turn out the most technologically perfect products, and to create new objective possibilities and conditions for a sharp increase in the rate of labor productivity growth. We know that Belorussia performs important tasks in social division of labor, in the area of development of machine building and instrument engineering, that is, those branches which predetermine in large measure a rise in the overall technological level of the entire national economy, furnishing it with the most sophisticated machinery, equipment, and technologies. Unfortunately at the present time many of our machines and instruments are inferior to the world's best. Sometimes their designers implement engineering solutions which are not in conformity with the requirements not only of tomorrow but of today as well. Belorussian large-payload trucks, for example, which are extensively employed in the extractive industries and which produce great economic effect, still contain a number of weak points. Our plans should focus scientists, designers and process engineers toward correcting design and other deficiencies in equipment. There is also a need substantially to extend the service life of Belorussian tractors, to increase time between overhauls on vehicles built by the Minsk and Belorussian Automotive plants, to reduce the weight and improve the reliability of the Gomsel'mash KSK-100 forage harvester. Considerable success has been achived by Belorussia's machine tool builders, who today are producing one out of every eight machine tools built in this country, but they too need the assistance of scientists in increasing the precision of machine tools and in equipping them with numerical-control devices. Particular attention should be focused on expanding research and design engineering projects connected with achieving total automation of production processes by employing various manipulators, industrial robots. This new and promising area of scientific and technological advance will make it possible, on the basis of advances in electronics and computer hardware, to achieve a genuine revolution in mechanization and automation of many manual operations in all branches and sectors of the economy. Research and development plans should give the "green light" to the development of industrial robots of the most diversified functions. Our economy, as was emphasized at the 26th CPSU Congress, should be economical. For this reason the search for fundamentally new ways and means of achieving more efficient, more economical utilization of resources, especially labor and fuel-energy resources, is exceptionally important both for science and for economic practices. Our republic, for example, each year consumes 32 million tons of standard fuel, and this consumption figure will increase substantially in the future. This indicates how critical is the problem of planning a profoundly well-conceived energy program extending many years into the future. Of course under present-day conditions one cannot count on success in solving these and other problems of the nation's economy without considerable improvement and a rise in the level of planning and management of scientific and technological advance, without rigorous, unswerving implementation of the instructions of Leonid Il'ich Brezhnev that "...management and, first and foremost, planning activity should be focused on the end economic effects." Certain experience in this important area has already been amassed in Belorussia. The Academy of Sciences, for example, was one of the first in the country to disseminate specific-program methods of planning basic research. Its initiative has been approved by the USSR Academy of Sciences. Our academy also extensively follows the practice of drawing up combined scientific research plans with large enterprises and associations in this republic, in which all stages are covered -- from initial research to practical adoption.

[Question] In connection with this, what in your opinion should be done to ensure that integration of science and production becomes more closely knit?

[Answer] I want to state that such a new, highly promising form of cooperation between scientists and people in industry as educational-scientific-production associations originated in Belorussia. The first such alliances with people in industry were established by the Belorussian Polytechnic Institute, which set up associations with the Minsk Automotive Plant and the Minsk Tractor Plant. At the present time there are more than 30 such educational-scientific-production associations in Belorussia. Scientific-production and scientific-technical associations have been established in our republic in the various branches and sectors of the economy, which make it possible to carry out the entire project cycle -- from conduct of research, development of industrial process, design of instruments and equipment, testing of models, to their extensive adoption in the branch or sector. The positive experience of such an association as Avtotranstekhnika, for example, in bringing new equipment and advanced technology on-stream has been recommended for adoption by other Belorussian ministries and agencies as well. Our plans for development of science and technology in the 11th Five-Year Plan and beyond call for establishment of complete-cycle scientific-technological complexes of interbranch orientation. Such complexes will make it possible to a substantial degree to optimize the functional structure of this republic's scientific-technical potential and to boost the practical return on research activities. One example of this is establishment in 1980 of what is perhaps not only the republic's first but also the nation's first interbranch organization — the Belorussian Republic Powder Metallurgy Association. This new organization, in addition to performance of its basic tasks, provides additional coordination of all scientific research activities conducted within the system of the USSR Ministry of Higher and Secondary Specialized Education and within this republic in the area of powder metallurgy and protective coatings. Now the Scientific-Production Association of Powder Metallurgy, jointly with interested enterprises, is drawing up a program of preparing for the manufacture of parts from metal powders, which will be produced at a plant which is under construction in Molodechno. Preliminary calculations have indicated that movement into production of the planned product list of such parts will make it possible to free approximately 500 metal-cutting machine tools and approximately 3,000 workers, and to save 20,000 tons of rolled stock. Anticipated savings will total 30 million rubles. Practical experience convinces us that it is also necessary further to expand the network of scientific-production associations, which play an important role in improving management of scientific and technological progress and which assist in retooling of industry and more rapid transition by the economy to the road of intensive development.

[Question]. What factors involved in boosting the level of scientific research planning are the most important at the present time?

[Answer] More extensive utilization of the specific-program method. It aims at precise formulation of tasks, selection of optimal ways and means of accomplishing them, and organization of efficient management of the research process and an effective system of monitoring research activity. Improvement in research planning practices, concentration of scientific manpower and resources in the most important areas of science and technology, and close coordination between current and long-range planning create the greatest possibilities for increasing the effectiveness of scientific quest and acceleration of utilization of research results. In the course of the scientific and technological revolution scientific discoveries as a rule are running increasingly ahead of practical application, and this is natural. Science is called upon to gain an increasing lead on production. For this reason effectiveness in planning the development of science and technology at the various levels becomes sharply improved when it is based on scientific and technical forecasts. A well-organized system of scientific and technical information, the role of which is steadily growing, is necessary in order to ensure that scientific and technological advances are made available to planning agencies and economic organizations. It has been established by science and confirmed by practical experience that when information service is well organized, the effectiveness of scientific research becomes substantially greater, and less time is expended by specialists in performing research and design activities, with a considerable increase in the quality of work performed. For this reason the 26th CPSU Congress pointed to the necessity of enhancing the role of scientific and technical information and the quality of forecasts with the aim of improving planning of the development of science and technology at various levels. We expect that, discussing current problems of improving planning of scientific and technological development, the participants in the scientific and technical conference will analyze the existing planning system and methodology, will present specific proposals and recommendations to improve this system, and thus will make a new contribution to the cause of accelerating scientific and technological advance in light of the resolutions of the 26th CPSU Congress.

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# COORDINATING ROLE OF TAJIK ACADEMY OF SCIENCES DESCRIBED

Dushanbe KOMMUNIST TADZHIKISTANA in Russian 14 Mar 81 p 2

[Article by V. Tsarikayev, scientist-secretary of the coordination council of the presidium of the Tajik SSR Academy of Sciences and a candidate of engineering sciences: "The Bridge Between Science and Production: Putting Decisions of the 26th CPSU Congress into Practice"]

[Text] The function of coordinating all scientific work in the republic has been entrusted to the Tajik SSR Academy of Sciences. To effectively manage research, the orbit of which draws in tens of scientific institutions and higher educational institutions and thousands of scientists, is to determine the strategy for scientific investigation and the way to accelerate scientific-technical progress -- a complicated matter.

It is more comforting to note that serious changes for the better have taken place. The methods and forms that are being applied have been improved. The effectiveness of science has been increased significantly. Owing to broad use of the newest technology, computer technology most of all, average research time has been reduced.

In the solution of basic problems in coordination, a large role is played by the planning of scientific-research projects. Cooperation and concentration of efforts are incorporated in coordinated plans. As a rule, they provide for conducting exploratory projects, fundamental and applied research, laboratory developments, and the introduction of scientific and technical achievements into practice.

This year, over twenty coordinated plans are in operation.

They provide for research on 454 topics and 112 problems (in 1980 there were 352 and 108, respectively). They are being done through the combined efforts of the 47 scientific institutions and higher educational institutions of the republic. Coordination embraces virtually all institutes and organizations that do research in the natural and social sciences.

Coordinated plans permit fuller and higher-quality use of the scientific potential of the republic for the further development of fundamental and applied research and the introduction of its results into production. In this connection, the basic attention is given to the accomplishment of tasks that have great economic value.

Analysis of coordinated plans for various years provides a basis for concluding that research is becoming more ponderable. At the same time, petty and non-urgent topics are being cut back with regularity. The subject matter of joint research by academy and ministerial scientific-research institutes and higher educational institutions is growing.

The basic work on these plans is accomplished by coordinating bodies, with a network that is continuously growing. Nine problem-councils and twelve coordination sections now are functioning under the Academy of Sciences. They are concentrating the efforts of scientists on the solution of the most important problems and are broadening direct contacts with departments and problem laboratories of higher educational institutions and with units of ministerial scientific-research institutes. Also personal business relations among individual scientists are being strengthened. This yields good fruits. Successes in this work have been achieved by problem councils and coordination sections that are headed by Academicians R. B. Baratov, S. A. Radzhabov, M. N. Narzikulov, B. I. Iskandarov, and A. A. Adkhamov of the TaSSR Academy of Sciences.

The 26th CPSU Congress stressed the importance of improving the coordination of scientific research. It was noted that wider use should be made of special-purpose complex programs as organic integral parts of state long-range plans for economic and social development and that there should be an increase in the validity and direction toward final results and the solution of concrete scientific-technical, economic, and social problems.

How have we organized special-purpose programs? Much is being done. Academy institutions have completed drafts of six special-purpose complex programs for the solution of scientific-technical problems. The programs include such important republic problems as protection of metals from corrosion, practical solar energy research, intensification of cotton raising, preservation and increase in fishing resources, the creation of new effective drugs on the basis of medicinal plants of Tajikistan, and the development of effective methods for working with Tayloriosis in large-horned cattle.

Draft special-purpose complex programs have been submitted to the republic Gosplan to be prepared for approval by the TaSSR Council of Ministers.

Scientific institutions of the Academy of Sciences also participate as joint performers in the development of a number of republic special-purpose complex programs. Each of them is a complicated complex of organizational, technological, economic, and social measures. All stages for program implementation have been provided for, beginning with the formulation of a scientific idea and ending with the utilization of research results.

The tasks of a number of special-purpose programs have already become an integral part of the State Plan for the Economic and Social Development of the Tajik SSR for 1981.

It is important to provide for the fulfillment of special-purpose complex programs in the planned time-frames and in full measure. Projects to implement the programs will be conducted by institutions and enterprises of various branches of the economy and will be under the management of head organizations. The unification of efforts on the basis of a concrete program is proving itself fully. Success will be aided also by the decision of the management of the Academy of Sciences and republic Gosplan to provide guaranteed financing to support special-purpose programs. First priority will also be provided for personnel and for supplies and equipment.

In accord with the Basic Directions for the Economic and Social Development of the USSR for 1981 to 1985 and for the Period to 1990, the development and implementation are planned for a large complex program relating to the formation and development of a South Tajik Regional-Production Complex. The head developer of this program is the republic Academy of Sciences' council for the study of the productive forces.

It would be incorrect, however, to consider that there are no deficiencies or unsolved problems in the activity of the coordination council. There are some and they are large. Scientific efforts and the supply and equipment resources are still poorly concentrated on the most important areas that open conceptually new routes and possibilities for building up scientific-technical and economic potential and that permit the creation of the engineering and technology of the future.

Not all problem councils and coordination sections are coping with the tasks that have been placed on them. Thus, for a long time, there has been a lack of activity by the problem council on "Feed Production and Pasture Improvement."

It must be stated that a number of councils see coordination functions as the registration of a large number of topics virtually unrelated to one another. There are still many petty topics in mathematics and in a number of areas of biological and social sciences. The problem council on "The Biological Foundations of the Exploitation, Conservation, and Protection of the Animal World" has not rid itself of petty topics. At times, a topic is worked on by only one or two specialists; this lowers the effectiveness of scientific research and leads to the scattering of labor, physical, and financial resources.

Activity is unsatisfactory in eliminating duplication in the conduct of scientific-research work. Such a phenomenon is witnessed particularly in biologically-oriented institutions. Patent search is poorly done on the topics that are coordinated, and few inventions are created on the basis of joint subject matter.

Little success has been achieved, unfortunately, in effective organization of cooperation between science and production. The results of research done according to coordinated plans is being realized slowly. Here, the possibilities are far from being exhausted.

The realization of developments in production is a task not only for academy institutes and higher educational institutions. Greater activity and initiative should be displayed by ministries, agencies, and enterprises which, like no one else, ought to be interested in the most rapid introduction of the results of research into production. But frequently, agency barriers stand in the way of innovation. It can only be in the best interests of both sides to build a bridge between science and production.

It is no secret that sometimes difficulties in introducing new technology arise also through the fault of scientific workers. Some projects are not implemented because of the low technical-economic level of the results achieved. Most often this takes place with researchers who work alone. Special-purpose complex programs are exactly what will open a broad road for putting developments into practice. They connect all the links of the chain: fundamental research, applied projects, experimental developments, and industrial production.

Today, the republic possesses a great scientific-technical potential: the number of scientific workers has increased, technical equipment for research has risen to a high new level, the physical base has been expanded significantly, and the number of personnel with high qualifications has grown. All this is a basis for very rapid integration of science and production and for putting the decisions of the 26th CPSU Congress into practice.

9645
CSO: 1814/34

## BSSR ACADEMY OF SCIENCES PRESIDENT ON REGIONAL SCIENCE AND TECHNOLOGY RESEARCH

Minsk ZVYAZDA in Belorussian 19 Apr 81 p 3

[Article by M. Barysevich, president of the BSSR Academy of Sciences: "On the Front Lines of Progress"]

[Text] This country's scientists are celebrating Soviet science day for the third time now. Establishment of Science Day is one more acknowledgement of its role in modern society. Science was assigned exceptionally important tasks by the 26th CPSU Congress. Leonid Il'ich Brezhnev stated in the CPSU Central Committee Report: "The conditions in which the national economy will be developing in the 1980's make acceleration of scientific and technological advance even more essential.... The Communist Party proceeds from the position that it is absolutely impossible to build a new society without science."

The Principal Directions of Economic and Social Development of the USSR in the 11th Five-Year Plan and the Period up to 1990, ratified by the 26th CPSU Congress, specify those scientific problems on which the efforts of Soviet scientists should be concentrated. If I were now to commence describing the principal areas of basic research in which Belorussia's scientists should work, I would list all those enumerated in the Principal Directions -- with the exception of thermonuclear and space research -- in the section pertaining to the development of science and acceleration of technological advance. And even in these two areas some problems are being solved with the participation of this republic's scientists. This indicates, first of all, that Belorussian science is an inseparable component part of Soviet science as a whole, and secondly that our scientists are working primarily on solving problems of pressing urgency.

At the same time there exist many regional problems which are specific to Belorussia, resolution of which should be handled for the most part by this republic's scientific manpower. Comrade Ts. Ya. Kisyaleu, presenting the CPB Central Committee Report at the 29th CPB Congress, correctly noted that academic instututes and higher educational institutions are not devoting adequate attention to investigation of problems of a branch/sector and regional nature. In the 11th Five-Year Plan this republic's research establishments should focus considerable efforts on elaboration of the scientific foundations of intensification of agricultural production, rational and efficient utilization of water resources, natural raw materials, the creation of new types of combined fertilizers based on peat, and study of the geologic structure of Belorussia with the aim of finding new deposits of commercial minerals. We must work more effectively on solving the problem of protection of the biosphere, and more vigorously elaborate the scientific principles of comprehensive utilization of the natural resources of the Polesye depression, excluding the possibility of irreversible disturbance of the ecological balance in this region.

We shall discuss separately the drafting of a republic comprehensive program of scientific and technological advance and its socioeconomic results over a period of 20 years (by five-year periods). It should formulate the major republic and branch/sector scientific and technical problems and should fully reflect the results of scientific research which will be utilized in production in the next five years and further in the future. This program should become the basis for planning scientific and technological advance in Belorussia.

Elaboration of a comprehensive program of scientific and technological advance is assigned to the BSSR Academy of Sciences, Gosplan, and Gosstroy. The Institute of Economics has been designated the lead organization in the academy. The first version of a program was drafted in 1980. Experience indicated that close cooperation between academic and branch science, science at the higher educational institutions, active participation by the republic ministries, state committees and agencies, as well as large branch production associations is essential in order successfully to accomplish this task.

Science is becoming a direct productive force in the era of the scientific and technological revolution. However, this is occurring only when the achievements of science are implemented in means of production and industrial programs, in the creation of material goods. A number of measures have been carried out in this republic in the last decade which promote acceleration of technological advance and strengthening of the link between science and practical application. The Central Committee of the CPB and the BSSR Council of Ministers have issued exceptionally important decrees aimed at raising the level of coordination of basic and applied research and accelerated adoption into production of scientific research results. Of great importance were the resolutions of the 19th Plenum of the CPB Central Committee, which was specially dedicated to the problems of scientific and technological advance in this republic. On the decision of this plenum, a commission of the Presidium of the Belorussian SSR Council of Ministers was established to deal with problems of scientific and technological advance. An important role in speeding up this advance should be played by execution of seven republic specific combined programs and 43 programs on major scientific-technical and economic problems, which have been ratified for elaboration in the 11th Five-Year Plan.

Much attention is devoted in this republic to the search for new forms of integration of science with production. A republic scientific-production association for powder metallurgy has been established, and the decision has been made to establish another similar association. Such scientific-production associations as Avtofiztekh, which includes the BSSR Academy of Sciences Physics and Technical institutes and the enterprises of Belavtomaz, the Minsk Tractor Plant-establishments of the academy physical-technical department Association, and an association which includes enterprises in the city of Gomel' and several BSSR Academy of Sciences institutes are successfully operating on a volunteer basis. A Republic Scientific-Technical Center for Hardening Technology was recently established. This center, in addition to academic institutes and industrial associations in the city of Minsk, includes the Scientific-Production Association for Powder Metallurgy and organizations of the State Committee for Supply of Production Equipment for Agriculture. Thirty educational-scientific-production associations have been established on a volunteer basis within the BSSR Ministry of Higher and Secondary Specialized Education system, including BPI-MAZ, BPI-MTZ, MRTI-NDI EVM -- the Minsk Computer Equipment Production Association, and others.

An important role in boosting the practical yield of science is played by academic establishments set up in the oblast centers of this republic, as well as dual-subordination subdivisions.

The Academy of Sciences has begun transitioning more extensively from contact with individual enterprises to direct links with ministries, agencies and large associations. In the 10th Five-Year Plan we were working on common plans with several union ministries, the BSSR Ministry of Industrial Construction and Ministry of Construction Materials Industry, with the Belorus'kaliy Production Association, etc. Cooperative programs with those all-union ministries with which we have worked in the past have been drawn up for 1981-1985 or are in the drafting stage, as well as with the USSR Ministry of Machine Tool and Tool Building Industry, Ministry of Non-Ferrous Metallurgy and Ministry of Ferrous Metallurgy and others, with BSSR Gosplan, and with the Belorus'kaliy, Integral, and BelOMA associations.

A plan is being drafted for joint scientific research efforts by establishments of the BSSR Academy of Sciences, USSR Academy of Medical Sciences and the BSSR Ministry of Health, which will be discussed and ratified in May at a joint session of the presidiums of the USSR Academy of Medical Sciences and BSSR Academy of Sciences and the board of the BSSR Ministry of Healt . The Presidium of the BSSR Academy of Sciences, the western department of the All-Union Academy of Agricultural Sciences imeni V. I. Lenin, and the BSSR Ministry of Agriculture are planning to draft a combined program of joint research by establishments of the BSSR Academy of Sciences and institutes of agricultural specialization.

Due to measures taken in this republic to speed up scientific and technological advance in the 10th Five-Year Plan, savings from direct practical adoption of scientific research results totaled 890 million rubles. One can cite many examples of extensive and effective application of research results generated by the institutes of BSSR Academy of Sciences, higher educational institutions, branch scientific research institutes and design offices of this republic in industry, agricultural production, and health care. There is a large number of exceptionally costly advanced innovations, however, work on which has gone on for years without practical adoption. What are the reasons for this? As noted in the Central Committee Report to the 26th CPSU Congress, this is due in large measure to imperfections in the economic accountability system, whereby it is unprofitable for enterprises to produce less expensive goods, because this worsens their production performance figures. It frequently happens that in developing new equipment clear technical and economic superiority over existing equipment and technology is not secured. Rapid adoption of new innovations, however, is harmed to an equal degree by subjective factors. Some managers talk a lot about technological advances but do little to achieve actual acceleration of progress. In many cases there is little executive responsibility and discipline both on the part of representatives of science and the practical experts. Accomplishment of a production target even with items already in full production requires considerable effort. But when a totally new product or manufacturing process is being brought on-line, one cannot work with conventional methods. A high degree of enthusiasm and conscientiousness is required of the executing personnel, as well as precise work organization and daily monitoring.

It was noted at the 26th CPSU Congress: "It is necessary to increase the personal demandingness on officials of agencies, enterprises, and institutes for the manufacture of new products and adoption of new, advanced technology. It is essential to eliminate everything which makes the process of adoption of the new difficult and painful.... Accomplishment of this task is of course no simple matter, requiring that one depart from obsolete ways and indices. But it is extremely necessary to the country, to the people, and to our future." Scientists must proceed from these instructions of the 26th CPSU Congress when planning basic and applied research and in practical implementation of research results.

As calculations indicate, in the future the level of return yielded by Soviet science must be increased from three to fourfold, with a doubling of scientific potential and a 50-70 percent increase in personnel. In the last 20 years such an increase in return was secured by a fivefold growth in potential and between a six and sevenfold increase in the number of scientific cadres. It follows from this that intensification of science and increased effectiveness of research are now primary elements. This can be accomplished only by raising the level of research scientist cadres, by creating a more flexible organizational structure of science, and by substantially strengthening its material and technical base.

Last year 38,000 scientific workers were employed in this republic, 3,130 graduate students and 176,000 undergraduates were enrolled at higher educational institutions. We have achieved these figures because the growth rate in the number of persons in these categories has been rising more rapidly in Belorussia in the last decade than in other republics. We are still, however, significantly behind the national level as regards percentage of doctors and candidates of sciences among scientific workers. Just as in the past, at some branch research institutes and higher educational institutions, a substantial percentage of laboratories and departments are headed by persons who lack academic degrees. In spite of this fact, the capabilities of the BSSR Academy of Sciences in the area of training candidates of sciences for this republic are being very inadequately utilized. But the most difficult problem is that of achieving a significant increase in the number of doctors of sciences. In the BSSR Academy of Sciences, which has more than enough candidates of sciences, there is an acute shortage of doctors of sciences in a number of areas of specialization. Thanks to measures which have been taken, approximately 200 doctoral dissertations are to be presented and defended by Academy personnel in the 11th Five-Year Plan. Other scientific establishments should also create conditions for increasing the number of doctors. The Academy of Sciences could lend consultative assistance and offer its laboratory facilities for the conduct of experimental research.

There has always existed a dialectical conflict between continuous renewal of the problems facing science and the sluggish mobility of its organizational structures. This conflict was resolved comparatively easily during the period of extensive development of science, when a new establishment or structural subdivision could be formed for each new area. Now this path is becoming rather the exception than the rule, while new scientific problems are arising with increasing frequency. It is necessary to resolve the question not only of what new problems should receive the efforts of scientific teams, but also a substantially more complex question: at the expense of what previous subject matter can this be done? Therefore the system of scientific research, as noted in the Central Committee Report to the 26th CPSU Congress, "must be considerably more flexible and mobile and not tolerate laboratories and institutes which fail to generate results."

Scientific facilities have become greatly strengthened in this republic in recent years. New laboratories and classroom buildings have been constructed, and design offices with experimental production units have been set up at many scientific establishments, which provide for the development of basic science and accelerate the practical adoption of scientific research results. At a number of establishments, however, laboratory and experimental design facilities are at a poor level. Solving new, exceptionally complex problems will demand a more solicitous attitude toward the needs of science.

Soviet scientists fully and enthusiastically support the Peace Program announced at the 26th CPSU Congress. They endorse the proposal to form an authoritative international committee which would demonstrate the vital necessity of averting a nuclear catastrophe. In the present situation imperialist strategists are counting increasingly more heavily on utilizing scientific and technological advances to further the arms race. They are increasingly channeling the scientific and technological revolution toward creating sinister weapons for the mass destruction of human beings and whipping up a war hysteria, at achieving worldwide economic and military domination. Under these conditions the question of increasing the effectiveness of our societal production on the basis of all-out utilization of the achievements of the scientific and technological revolution is a key problem not only of development of the socialist society but also of all human civilization.

We can state with assurance that the scientists of Belorussia, marching shoulder to shoulder with the teams of Soviet science, are taking most active part in carrying out the principal tasks assigned by the 26th Congress of our glorious Leninist Party and which are aimed at securing the building of communism and strengthening peace on earth.

3024
CSO: 1811/54

## ESTONIAN SCIENCE INSTITUTES PRODUCE TOO FEW INVENTIONS

Tallinn RAHVA HAAL in Estonian 10 Jun 81 p 2

[Article by Heino Koitel: "Invention and Technological Progress"]

[Text] The scientific and technical revolution has given birth to inventions, scientific research and discoveries, and their application in the economies of all developed countries. The effectiveness of these activities determines the scientific-technical and industrial potential of a country. The application of inventions and scientific breakthroughs was strongly emphasized during the 26th CPSU Congress. It deserves mention that our republic claims an average of one invention in less than every 10 minutes. Each year, dozens of scientific discoveries of international acclaim are registered in this country. The latest, registered as No. 246, on the nature of high-temperature photoluminescence of crystals, was authored by Academician K. Rebane, corresponding member V. Hiznjakov and P. Saari.

More than 300,000 inventions, 100,000 of which were applied in industry, were registered in the USSR during the 10th Five-Year Plan. The economic effect of inventions and the application of labor-saving techniques exceeded 7 billion rubles. Soviet inventions are patented in dozens of countries abroad; approximately 50 countries have bought their licenses.

During the last few years, our republic also has made certain progress in the area of inventions and in patenting-licensing activities. Our innovators have captured six first prizes in the all-Union socialist competition. Their efforts contributed to a saving to the national economy of almost 330 million rubles during the 10th Five-Year Plan. Over 1,000 inventions were registered in the republic during the five-year period. More than 60 inventions in our republic now have been patented abroad; 200 or more foreign patents have been issued. Several licenses have been sold to foreign firms. Licenses have been sold by the Tallinn Polytechnic Institute, the Special Design and Technical Office "Desintegrator," and the Silicon Concrete Research Institute. The first scientific discovery has also been registered in our republic. The above clearly demonstrates that serious work is carried out in the field of inventions.

Despite an environment basically favorable to scientific and technical innovation, numerous shortcomings and problems exist, which beg solutions.

The savings attributed to our innovators during the 10th Five-Year Plan have been considerable; however, it must be stressed that most of the savings resulted from proposed labor-saving production methods. Invention-related savings were negligible. It is a well-known fact that inventions and basic research are a precondition to progress in science and technology. The institutions and enterprises of our republic have during the last few years registered an average of 200 inventions per year. That is a small number. The Latvian SSR, for example, registers over one thousand inventions each year. When we compare the number of inventions with the scientific and technical potential of our republic, the following picture emerges. There are a few thousand scientific and technical personnel in our republic; of this number, over 130 are doctoral candidates and about 1500 are masters degree candidates. These people work in fields which are conducive to and demand research and invention, e.g., engineering, sciences, physics, chemistry, medicine, etc. However, their contribution to innovation is negligible. Based on their qualifications, the majority of above-mentioned professionals should be capable of working out inventive solutions to problems in science and technology. The total number of inventions in our republic would increase considerably, if one-tenth of our science and engineering personnel would author at least one invention per year. Such a task seems quite realistic. It should be of interest that almost half of the inventions within our republic are attributed to innovators at the Tallinn Polytechnic Institute and the Radio Electronics Design Office. The number of innovations by the Academy of Sciences of the Estonian SSR as well by other higher educational institutions (The University of Tartu), is considerable. Thus we can see that other institutions, design offices and enterprises do not contribute significantly to this effort. It is imperative that the management of the latter establishments show increasing interest in the areas of patenting and invention.

The introduction and adoption of scientific and technical breakthroughs and innovations presents a most acute problem. An average of one-third or one-fourth of all inventions are adopted in industry, and then by only a few enterprises. Serious efforts should be directed toward the application in mass production of the most significant innovations. Recently, a new organization "Efekt" was established in our republic primarily to promote the rapid adoption of inventions in the national economy.

The protection of trade-marks and industrial design should receive greater attention than in the past. The majority of our enterprises have a registered trade-mark; however, many trade-marks no longer meet today's requirements. There should be a bold new move toward the use of descriptive rather than graphic trade-mark design.

Our republic has achieved a first in the protection of industrial design: in 1966, the first dozen or so industrial designs in the USSR were registered in the name of "Estoplast." In this area, we have regrettably lost our leadership. The following is difficult to explain: The Industrial Arts Department of the Institute of Fine Arts has not applied for design protection for any of the dozens of interesting product designs developed by the department.

The improvement of the qualifications of patent officials seems an effective means to increasing the efficiency of patent and licensing procedures. This effort has been enhanced in our republic by the establishment, several years ago, of a branch of the Moscow Central Patent Institute. Only four similar branches of the Institute exist within the USSR. Unfortunately, not every enterprise has shown great enthusiasm toward developing the skills and expertise of their patent specialists. Courses in the fundamentals of patent procedures should be introduced in higher educational institutions throughout our republic, as well as in the Institute for Improving the Qualifications of Managerial Personnel and Specialists in the National Economy of the Estonian SSR. It seems highly abnormal that engineers, lawyers, and economists with advanced degrees lack the basic knowledge of patent procedures.

The establishment of a Patent Department within the Institute of Information will certainly add a new dimension to the development of patent-licensing activities of the republic. This department will coordinate and assume overall responsibility for patent assignments throughout the republic.

The timely solution of problems discussed above will hopefully bring about a rise in productivity and foster rapid progress in science and technology throughout our republic.

7706
CSO: 1815/3

## NEED TO BROADEN SOCIOLOGICAL STUDIES NOTED AT ACADEMY OF SCIENCES SESSION

Kiev PRAVDA UKRAINY in Russian 1 Jul 81 p 2

[Article by V. Shinkaruk, director of Institute of Philosophy of the UkSSR Academy of Sciences: "Toward a Close Alliance"]

[Text] Speaking at a session of a general meeting of the Academy of Sciences on 25 March 1981, member of the Politburo of the CPSU Central Committee, first secretary of the Central Committee of the Communist Party of the Ukraine Comrade V.V. Shcherbitskiy pointed to the need of "conducting on a wider scale and using sociological research, which in its turn requires the development of suitable methodologies."

In recent years there have been created at the Institute of Philosophy of the UkSSR Academy of Sciences new sociological subdivisions; promising directions in the field of sociology of labor and management, social-vocational orientation of youth and the study of public opinion have been fruitfully developed. The Ukrainian Section of the Soviet Sociological Association, whose base organization is the Institute of Philosophy, is doing major coordinating and methodological work. We possess rich traditions of conducting sociological studies at the L'vov Television Plant in the Kiev section of the YuZZhD [Southwestern Railroad (?)], the Odessa Sewing Associations imeni Vorovskiy and at a number of enterprises in the city of Kiev, in rural rayons of the republic and so on. Since 1979, since 1979, the institute has been conducting comprehensive research on improving the style and methods of management at the Black Sea Shipbuilding Plant. This work is continuing the research which we started at republic enterprises toward the end of the '60s. The cooperation of the institute with the plant significantly expands the experimental basis of approval and introduction of scientific ideas.

Our task is to improve the effectiveness of sociological research as a source of informational and scientific-methodological assurance of accomplishment of tasks of social control proposed at the 26th CPSU Congress.

The congress emphasized with new strength that under present-day conditions the indicator of the effectiveness of social knowledge is the degree of its inclusion in the practical work of social production. It is clear that the problem in particular consists of a search for effective forms of combining the achievements of the social sciences (sociology, social psychology, ethics, pedagogy and the rest) with the practical work of management of production collectives. In our view, significant assistance on this plane could be provided by the creation at large enterprises, associations, sectors and regions with the cooperation of academic institutes and departments of VUZ's of consultative methodological centers (CMC) for social and social-psychological problems of management. By way of an experiment, such a center on the initiative and with the participation of the Institute of Philosophy of the UkSSR Academy of Sciences has been established at the Black Sea Shipbuilding Plant.

We believe that the CMC is an effective organizational form for the accumulation of social learning and advanced experience and for bringing them to directors of all levels and utilization in the practical work of social management.

A CMC arranges propagandization of sociological and social-psychological methods of production management, acquaints people with advanced experience and modern practice of management, arranges individual and group consultations for heads of production collectives, carries out sociological examination of proposed (and even already introduced) innovations and provides forecasts of the possible social consequences of these innovations. It provides methodological and practical aid in the working out of programs and conducting sociological research and social experiments aimed at raising production efficiency, promotes the finding of social reserves for stabilization of the cadre composition of the collective, puts out proposals and recommendations for social-vocational and production adaptation of different categories of workers and so on.

The first results of the work of the CMC at the Black Sea Shipbuilding Plant show the fruitfulness of the proposed form of cooperation between an academic institute and an industrial enterprise in the solution of current and long-term problems of social-economic and ideo-educational and moral development of labor collectives.

I would like to point out that such kind of work not only strengthens our ties with practice and permits investigators to feel the hot pulse of production life. They fertilize the theory itself, stimulate the development of creative thought and, in the final analysis, contribute to the growth of new knowledge.

We believe that the demand for sociological studies will grow in the future. At the same time there are extremely few professionally trained sociologists in the republic; their status has still not been clearly determined. We think that the republic Gosplan, the State Committee for Labor and Social Problems and the Ministry of Higher and Secondary Specialized Education should take the necessary steps for training of specialist sociologists and development of sociological services.

Chairs of sociology have now been created at Kiev and Khar'kov universities. But the organization of these chairs does not solve the problem of training of graduate sociologists in the necessary numbers.

As pointed out in the materials of the 26th CPSU Congress, the development of science and technology in the 11th Five-Year plan must be still more subordinated to the solution of the most important problems of the further progress of Soviet society and

and the accelerated shifting of the economy to the route of intensive development. It is to be understood from this that strengthening of the ties of the social sciences with practice constitutes an important direction of intensification of the development of social production. It is namely this key problem that science and practice of social management are called upon to solve.

7697
CSO: 1814/41

# CHARGES OF PLAGIARISM RAISE SCANDAL AT UKRAINIAN GEOLOGIC INSTITUTE

## Internal Conflicts Over Charges

Moscow SOTSIALISTICHESKAYA INDUSTRIYA in Russian 18 Jan 81 p 3

[Text] Last year in August, the people's court of Radyanskiy Rayon of L'vov received petitions from three citizens living in different parts of the city but working together at the Institute of Mineral Geology and Geochemistry of Fossil Fuels of the UkSSR Academy of Sciences. All the petitions ended identically: "... I request that criminal proceedings be instituted against Viktor Fedorovich Polkunov... and that he be punished for wittingly disseminating slanderous information about me." Doctors of Science V. Kushniruk and V. Kolodiy and Candidate of Sciences V. Shchepak were the plaintiffs.

"The act is unprecedented," the legal experts declared in their distress. "Communists wish to settle an argument in court which began at a party meeting."

As we see, one could hardly imagine that the plaintiffs would have a poor acquaintance with the main provisions of the CPSU Charter, party ethics and morality. Nonetheless, the fact is that their petitions are at a people's court. And an explanation can be found for every fact, although at times it may be hard to do so.

It all began in 1971. True, the chronicles of the Institute of Geology and Geochemistry of Fossil Fuels (IGiGGI) of the UkSSR Academy of Sciences did not print anything special that year. But in the case of V. Kushniruk--the young candidate of sciences--who was not ordinary--his first monograph "Geologicheskoye stroyeniye i tektonicheskiye osobennosti L'vovsko-Volynskogo ugol'nogo basseyn" [The Geological Structure and Tectonic Special Features of the L'vov-Volynsk Coal Basin] was published.

This should have been a time for rejoicing, but suddenly a clap of thunder reverberated: L'vov geologists noted that the book was not entirely the authors's... here and there he had borrowed with light editorial cosmetic touches from the book "L'vovsko-Volynskiy kamenno-ugol'nyy basseyn" [The L'vov-Volynsk Bituminous Coal Basin], written by geologist D. Bobrovnik in coauthorship with a group of their colleagues and published back in 1962. But he copied out a large part, "shmat" as they say in the Ukraine, of an unpublished commercial geological account edited by M. Struyev and kept in the Ukrainian Territorial Archives. All this, of course, was without any references to sources and authors.

The institute's party bureau, on examining the personal file of communist V. Kushniruk, ruled:

"For violation of scientific ethics as indicated by the copying out of certain geological materials from archival and published works without citation of their authors, V.A. Kushniruk is to be reprimanded."

In order to understand what relationship these matters of days long past have to the story of the three petitions to the people's court, it will be necessary to familiarize oneself with an excerpt from a speech by senior engineer V. Polkunov (the complaints were namely in regard to him) at an open party meeting at the institute held 26 January at which the question was discussed of improving publishing and editorial work at the institute.

"Our editorial and publishing activities contain defects whose consequences it is difficult to predict. Thus, in 1978 there was published V.A. Kushniruk's monograph "Gazonosnost' uglenosnoy tolshchi L'vovsko-Volynskogo ugol'nogo basseyn" [The Gas Bearing Capacity of the Coal Bearing Stratum of the L'vov-Volynsk Coal Basin]..., many pages of which... had been borrowed from the collective monograph by D. Bobrovnik and coauthors as well as from other works without them being cited"....

And so, plagiarism again? It turns out that Doctor of Sciences V. Kushniruk has not at all parted with those work "methods" that Candidate of Sciences V. Kushniruk used? And the reprimand given him seven years ago at the party bureau has exerted no effect whatsoever? Moreover, "having sown his wild oats" and showing confidence in himself, Venedikt Alekseyevich went over from passive defense to a broad attack not only on the principles of ethics and morality but also on those who are attempting to find him guilty of unseemly acts. And here facts and counterarguments absolutely do not mean a thing to him. An example of this was his declaration at the party bureau. "On 26 December 1978, V.F. Polkunov called me a plagiarist in my absence at an open party meeting at the institute.

"I am surprised by the behavior of that unrestrained good-for-nothing Polkunov.... Who gave him the right... to insult me, to denigrate me before pupils and the institute's collective... Do I really deserve to have some unrestrained member of the collective (without a scientific degree) rate me as a scientific worker....

"I request the party bureau to investigate this individual, to punish him strictly according to party policy and to isolate him from our collective. I request the party bureau to oblige Polkunov to apologize at an open party meeting and to say who is prodding him to do this."

V. Polkunov made no attempt to apologize and to explain "who was prodding him to do this." The party bureau created a commission for elucidating the circumstances of the case; it, in particular, established:

"Certain coincidence are to be found in individual lines and sentences of V.A. Kushniruk and in works of other authors without those works being mentioned, which constitutes a violation of generally accepted norms of use of published literature."

Kushniruk was placed "on notice" and they immediately begun a discussion of the behavior of the disturber of tranquillity--Polkunov.

"Here you do not have a big criminal," G.N. Dolenko, directors of the institute, declared concerning the actions of V. Kushniruk, "here there is only the inability to use printed materials.... Polkunov is evidently a no-good person.... His principles are not party principles, but party myopia."

A little later, at a meeting of the party bureau, it was noted in regard to Polkunov --"to point out."

And could it possibly be that V.F. Polkunov is truly a"no-good person," who has lost sight of party principles? Let us see.

Viktor Fedorovich Polkunov, a CPSU member since 1945, is a veteran of the Great Patriotic War. By education, he is a geologist. He has to his credit scientific works and inventions that have been of bit economic benefit.

"A characteristic feature of the scientific activity of V.F. Polkunov," it is noted in a description signed by Professor V.I. Kityk, deputy director of the institute, is exhaustive and multilevel study of an investigated subject.... He has a great deal of experience in the supervision of the group entrusted to him. He actively participates in public life, serving as a non-salaried inspector on the Radyanskiy Rayon Committee of People's Control and as a member of a people's control group at the institute."

Does Polkunov have any defects? He does, like any other person. I would say that he is excessively hot-headed and there is a "bristling" aspect to his character.

Although he did not count on gratitude for his criticism, he did not anticipate that he would be transformed into an object of concentrated blows from the administration and the party bureau. "Materials were collected" on him; they sought out misjudgments in his behavior, suggested that he submit a resignation... Polkunov had to fight this off and assume a counteroffensive. His nerves were wound up.

V. Polkunov became a persona non grata not just because he had dared appear with a criticism. He provided an incentive for others.

Shortly after Polkunov, senior engineer Ye. Fedorov "mutinied." He accused his colleague V.M. Shchepak of having stolen a scientific idea of his and using it in his works.

Candidate of Sciences L. Gutsalo, whose elaboration was included in V. Kolodiy's "Karta prakticheskaya tsennosti dissertatsii" [Chart of the Practical Value of a Dissertation], came out with a protest.

Senior scientific associate A. Ivanov sent much factual material on the plagiarism to VAK [Higher Arbitration Commission].

A number of coworkers condemned the practice of "added" signatures, which has become frequent at the institute: personnel who are senior as to title and position put their own names under publications next to those of the real authors, without even asking for their consent.

The incidents were investigated. The institute's academic council and the party bureau created numerous commissions, which, on the one hand, pointed out certain violations and, on the other, toned them down. The commissions' conclusions were discussed at meetings of the academic council and the party bureau. But no one dared to say: "Comrades, could it not be that not everything is all right with us? Shouldn't we look into the matter and punish the guilty ones?" They did not go so far. "Insurgents" walked among the guilty ones. Academician of the UkSSR Academy of Sciences G.N. Dolenko, the institute's director. found for them the most appalling words. Their behavior was being discussed, their "locks were being picked" in an effort to cast a shadow on their reputation. One, it seems, was once seen "drunk," another with a woman on his arm at the Lychakovskoye Cemetery"....

The institute was in a fever, but the administration and the party organization saw as their task not as a struggle with defects but how to best pacify the dissidents.

Ye. Fedorov was threatened with being fired from the institute; so far he is "on notice."

It was more difficult with L. Gutsalo whose prestige goes far beyond the walls of the institute. But, as they say, could there be a man who... The following incident was put to use: once L. Gutsalo, while using compensatory leave, went to Moscow on his own to take part in a scientific conference. They found this sufficient to make a truant of the scientist and to prepare an order on firing him as charged.... Had not a legal advisor pointed out the illegal nature of the actions of the administration, L. Gutsalo would have been obliged to leave the institute.

Candidate of Sciences B. Chalyy was dealt with more simply. When the time approached for his reappointment to the position of senior scientific associate, the department and its head institute director G.N. Dolenko unanimously were in favor of having B. Chalyy remain at the institute. The party bureau, of which G.N. Dolenko was a member, was also unanimous, but when the time came to resolve the question at the academic council, the institute's director proposed... not to include B. Chalyy in the secret vote list. His motive? The subordinate did not share the views of the chief in regard to a scientific hypothesis. Such are party principles, such are scientific ethics!

Today Candidate of Sciences Boris Nikolayevich Chalyy is not on the roster of the institute. Likewise on the rosters of other institutions.

This enumeration could be continued, but it would seem that enough has been said.

Twice encountering the director, I tried to find out: what was the administration doing to improve the atmosphere of the collective. The first time Grigoriy Nazarovich gave a most laconic answer:

"We must get rid of Polkunov."

At the second meeting, his reply was somewhat more extensive:

"We must get rid of Polkunov and others like him."

That is all. You see, how simple things are! There is no need to deal with plagiarists, with people who "add names," with bootlickers, nor is there a need to carry out any sort of educational work among the collective.

So as not to be misunderstood, I want to immediately qualify; I do not set as my task to belittle the contributions of such a respected scientist as Academician G.N. Dolenko, nor to negate the achievements of the institute he heads. But somewhere along the line G.N. Dolenko made a mistake, which brought in its wake a whole series of errors. It would be difficult to state when this took place. Possibly, when he first supported V. Kushniruk, or when he shouted at the critical comments of a colleague, or when he thought that the party organization was his obedient helper. I do not know, I have no intention of passing judgment. I believe that the oblast party organs and the presidium of the UkSSR Academy of Sciences will look into this.

## Public Reaction to Scandal

Moscow SOTSIALISTICHESKAYA INDUSTRIYA in Russian 17 Jun 81 p 2

[Text] The report provoked a large flow of readers' responses. "You did well," A. Shkatov writes from Tyumen', "to bring V. Kushniruk' statement to the party bureau. One can see immediately from it what kind of a person you are dealing with. Only by letting the whole world know should you deal with such people..."

While sharing the point of view of the authors of such letters, still it is not possible to agree with their opinion that "only by letting the whole world know" is it possible to deal with negative phenomena. We believe that V. Serpukhovskiy of Tula, who writes: "I think that the institute's workers have been damaged much more by the fear of having the quarrel spread outside the house and by a lack of principled character," is a great deal more correct. "The conflict did not arise unexpectedly. It has smouldered for long years. This illness should have been treated (and cured!) long ago. By whom? By the party bureau and all honest scientists. They, no doubt, make up the majority at the institute...."

This thought is confirmed by facts contained in a letter by institute associate Plotnikov. "I have been working for about 20 years at the institute discussed in the article," he writes. "We have had talk about plagiarism and plagiarists for a long time; they possessed, so to say, a cultured character. The disease of plagiarism was created in open and latent form by an atmosphere in which free exchange of opinions concerning new results of research, concerning new thoughts was impossible... The abnormality of such a situation would appear to have been clear to everybody and to require immediate rectification. But when a person was found who tried to correct this situation, he found he had no support...."

Those who enter the fight against routine and stagnation, against violations of elementary ethical norms frequently find it difficult. Why conceal the fact--letters in the editors' mail are to be found that are permeated with pessimism. Assistant V. Baryshev from Magnitogorsk thinks, for example, that it is hard to be successful in the struggle with negative phenomena when a person is not vested with power or does not have to his credit ponderable services to society. A number of readers cite examples of their own failures. True, there are not many such letters. But replies to them suggest daily occurrence--and examples to that can be a great multitude, if you fight for it to the end.

Special interest is presented by letters whose authors analyze the roots of plagiarism and attempt to suggest measures for dealing with it. "Contemporary science has become a mass science." Yu. Ovcharenko, a candidate of sciences from the Ukrainian Scientific-Research Institute of Geological Prospecting (UkrNIGRI), writes. "But the aspect, as one would expect, is connected with an influx of the "nonoutstanding." The desire to have a life career causes some of these people to forget fundamental moral principles. It is namely they who cultivate the thought that unpublished scientific reports of other authors stored in archives ostensibly belong to no one. Therefore each one is free to deal with them as he sees fit.

At many institutes, the system is in practice where the leaders become, so to say, honorary coauthors of their subordinates. This is how a scientific "corvee" is legitimatized. The time is long overdue for working out rules stipulating the conditions of coauthorship of supervisor and subordinate. Without such rules, coauthorship is sometimes transformed into a concealed form of plagiarism, breaks down science from within and distorts the consciousness of young scientific workers...."

What was said by Yu. Ovcharenko is added to in his letter by L. Reyfman, a coworker from the same institute. "At the present time," he writes, "a very paradoxical situation has been created. Should the protection of authorship of printed works, weak though it be, still exist, then manuscript materials in no way come under the operation of protective measures. They constitute hunting land for compilers and plagiarists.... Such a situation does moral and actual damage to our science. This means that something must be changed. Possibly, the practice of deposition should be expanded or otherways sought. It would seem that lawyers should think of a more precise regulation of authorship protection in science because the worst law is the absence of law...."

Sharing in the existing way the concern of our readers, we hope that the All-Union Agency for Copyright will put in its formidable word, as well as the USSR State Committee for Science and Technology, lawyers and scholars.

Unfortunately, we did not feel the same anxiety in the answer of the presidium of the UkSSR Academy of Sciences signed by Academician of the UkSSR Academy of Sciences V. Trefilov, vice president of the UkSSR Academy of Sciences. Without denying that "recently an unhealthy atmosphere was created at the institute and its intensification has contributed in certain measure to inconsistent, unconstructive and, in some cases, incorrect actions and statements by the institute's director Academician of the UkSSR Academy of Sciences G. Dolenko in conflict situations," the reply asserts that the facts described in the article "Scandal in an Academic Family" "... are known to the leadership of the academy and have been the subject of an examination by a specially created commission, consisting of the leading scientists and personnel of the central organization of the UkSSR Academy of Sciences." What measures has the commission designated?

It says in the reply "that Doctor of Geological-Mineralogical Sciences V.A. Kushniruk did violate in a number of cases scientific ethics, expressed in the use of materials of other authors without appropriate citations and references." But all this, judging by the reply, are mere trifles, inasmuch as the borrowings were "insignificant in regard to its (the dissertation's--Ed.) fundamental content."

In a word, as is apparent from the reply, nothing special had occurred and the newspaper should not have bothered raising an alarm. The concluding lines of the reply are worded quite vaguely: "The presidium of the UkSSR Academy of Sciences has outlined measures for raising the level of supervirion over the fundamental sectors of activity of the Institute of Geology and Geochemistry of Fossil Fuels of the UkSSR Academy of Sciences and restoring the atmosphere in it." What these measures are and how they will be implemented remain a mystery.

The oblast's party organs reacted quite differently to the article in the newspaper. Here is what First Secreatry of L'vovskiy Obkom of the Communist Party of the Ukraine V. Dobrik wrote to the editors:

"In the article 'Scandal in an Academic Family,' published 18 January 1981, serious defects are disclosed in the operation of the Institute of Geology and Geochemistry of Fossil Fuels of the UkSSR Academy of Sciences.

"The article was discussed 19 February at a party meeting at the institute. The criticism was acknowledged to be correct. The meeting censured the existing atmosphere of liberalism at the institute in the assessment of the scientific value of the works of V.A. Kushniruk and other scientific workers, the violation of scientific and party ethics by some communists and the incorrect style of supervision by the director of the institute Academician of the UkSSR Academy of Sciences G.N. Dolenko; it pointed out the inadequate level of party control over the activities of the administration.

"On 4 March the bureau of the Radyanskiy Party Raykom of L'vov pointed out in an adopted decree to the bureau of the institute the low level of ideo-educational work among the collective and lapses in cadre work and charged that the existing defects be eliminated. For defects in supervision of the collective, selection and education of cadres, improper reaction to criticism and creation of an unhealthy atmosphere among coworkers, CPSU member, institute director Comrade G.N. Dolenko is given a reprimand with a notation in his record card. For violation of norms of party ethics communists V.V. Kolodiy and V.M. Shchepak are given reprimands.

"The case of communist V.A. Kushniruk will be examined later at the bureau of the party raykom (in connection with the illness of the latter) and the editorial board will be subsequently notified concerning it.

"The obkom of the Communist Party of the Ukraine directed the attention of L'vov Gorkom, the Radyanskiy Party Raykom and the party organizations of scientific-research organizations of the need of increasing ideo-educational work among cadres, raising of requirements on the level of scientific research and the creation of a creative atmosphere among collectives."

We believe that this reply will fully satisfy A. Moskalenko, a female worker from Voroshilovgrad, who writes in her letter: "I am not a scientific worker and am not acquainted with the fine details. But I know something else: life puts before us common demands, the chief of which are—objectivity, a party approach to work."

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# Translation

## TULAREMIA IN THE USSR

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25 October 1982

# TULAREMIA IN THE USSR

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# CHANGES IN PROPERTIES OF TULAREMIA PATHOGEN UNDER THE INFLUENCE OF ERYTHROMYCIN

Moscow ZHURNAL MIKROBIOLOGII, EPIDEMIOLOGII I IMMUNOBIOLOGII in Russian No 12, Dec 73 (manuscript received 12 Jul 73) pp 98-101

[Article by R. I. Kudelina, Tularemia Laboratory, Institute of Epidemiology and Microbiology imeni N. F. Gamaleya, USSR Academy of Medical Sciences, Moscow]

[Text] The highly virulent S form, which circulates in endemic tularemia sites, is the natural form of existence of Francisella tularensis. The R form, which has no virulent properties at all, like the intermediate SR form, appears when the S form is cultivated in the laboratory on artificial nutrient media [1, 3-6 and others].

We submit here data on changes in properties of the tularemia pathogen under the influence of erythromycin. This question had not been previously discussed in the literature.

Studies were conducted on models of the Schu strain (Nearctic race) isolated by Foshey in the United States from a patient with tularemia and strain No 543 (Central Asian race) isolated from a midday gerbil by Aykimbayev. Both strains had the typical morphological, cultural and biochemical properties (fermented glycerin; contained the enzyme, citrulline ureidase; caused death of white mice, guinea pigs, as well as rabbits in the case of the Schu strain, when isolated [a few?] microbial cells were given by hypodermic injection).

Unlike the Holarctic race strain, the above strains were sensitive to erythromycin in concentrations of 12-25 μg/mℓ [2].

Our study consisted of two main stages: production of erythromycin-resistant variants and comparative study of their morphological, cultural and biological properties. We used conventional methods in these studies: successive passages on solid agar medium consisting of fish hydrolysate with cystine, glucose and blood, with increasing concentrations of erythromycin. We performed up to 11 passages, and the maximum antibiotic concentration was 6400 μg/mℓ.

Morphological examination of the obtained variants under a light microscope failed to demonstrate appreciable differences. Electron microscopy by V. L. Popov revealed that the cells of erythromycin-resistant variants were larger than those of the original strains. For example, the dimensions of cells of the original Schu strain were 650×400 μm, for its variant they were 700×500 μm, for strain No 543--500×250 and 700×450 μm, respectively. It should be noted that, unlike strain No 543, the Schu strain was larger and had more marked polymorphism, which is consistent with the data of Olsuf'yev et al. [3] and Olsuf'yev [4].

When erythromycin-resistant variants were grown on coagulated yolk medium, we failed to detect any distinctions related to start and nature of growth. Differences were demonstrated only when the cultures were plated in dishes with agar from fish hydrolysate with cystine, glucose and blood. After 4-day incubation at 37° and subsequent storage of dish contents at 4° for 1 day, the original Schu and No 543 strains formed in the dishes convex, shiny, smooth, light-blue--whitish, opaque and large (2-2.5 mm) colonies, while the erythromycin-resistant variants formed flat, grayish, transparent small (<1 mm) colonies. In both instances, the colonies were homogeneous, occasionally differing slightly in dimensions, which depended on density of plating and thickness of the agar layer.

We plated a suspension consisting of a mixture of cultures of the original strain and erythromycin variant (Figures 1 and 2 [photos not reproduced]) in order to better compare the morphology of colonies of original strains and their variants.

Cultures of the original strains and their variants were inoculated in test tubes with blood agar in order to determine the minimum inoculation dosage and demonstrate differences in growth rate. For inoculation, we used 2-day cultures grown in a coagulated yolk medium, standardized in accordance with the bacterial standard of the State Institute for Control and Standardization of Medical Biologicals imeni Tarasevich to 1 billion microbial cells per milliliter. The inoculation dosage constituted 0.1 mℓ of different 10-fold dilutions of cultures of $10^8$ to 10 bacterial cells. We recorded colony growth daily for 9 days. Evaluation thereof was made on a 4-point scale. We failed to demonstrate differences in growth of cultures of original strains and variants when large doses of culture ($10^6$-$10^7$) were inoculated. Differences in intensity and time of appearance of growth were only noted with inoculation of doses of $10^5$ or less. For example, when a culture of original Schu strain ($10^5$) was inoculated, growth in the form of isolated colonies was recorded from the 1st day, and by the 3d day was rated as 3+ (profuse), whereas with inoculation of the same dose of culture of the erythromycin variant growth was demonstrable only on the 6th day (2+). The minimum inoculation dosage, with which growth was observed, constituted 10 microbial cells for the original Schu strain and $10^3$ for the variant.

We also observed less profuse and later growth in the variant of strain No 543, as compared to the original strain, although no differences were noted in inoculation dosage.

We tested virulence and immunogenicity of the variants in experiments on white mice weighing 12-15 g, which were infected subcutaneously by different 10-fold dilutions of the erythromycin variant cultures (1-100 million bacterial cells) and cultures of the original strains (0.1-1-10 microbial cells), using 3 mice per dosage. In all, each strain was used to infect 36 mice (27 with the variant and 9 with the original strain). We assessed strain virulence on the basis of time of death, pathological anatomy, accumulation of pathogen in the spleen, liver and blood of the mice, as well as results of cultivating the spleen on a coagulated yolk medium.

Virulence of the new variants diminished drastically, as compared to the original strains: when infected with variants, the mice survived after being given 100 million microbial cells, whereas after infection with the original strains they died on the 5th-6th day after injection of 1-10 microbial cells, and we demonstrated considerable accumulation of the pathogen in the internal organs.

Concurrently with decrease in virulence, the erythromycin variants also showed attenuation of immunogenic properties, and this was more marked in the variant of strain No 543 than the variant of the Schu strain. Thus, all mice infected with different doses of a culture of the No 543 variant died of tularemia 1 month after giving them 100 microbial cells of the original (homologous) virulent strain. The average time of mouse death was 5-7 days, depending on the dosage of the initially given variant. All mice given 1-100,000 microbial cells of a culture of the Schu strain variant died on the 5th-7th day after being given 100 microbial cells of the homologous virulent strain. We observed only partial death among mice infected with 10 and 100 million microbial cells of the variant culture after administration of the virulent strain (2 out of 3 infected mice died). Evidently, the erythromycin variant did not lose its immunogenic properties entirely.

Control mice, which were infected concurrently with the experimental ones, given 1-10 microbial cells of Schu and No 543 died of tularemia on the 5th-7th day.

We studied the antigenic properties of the produced variants using the reaction of agglutination with rabbit sera obtained by immunizing animals with cultures of virulent strain No 503 (S form) and attenuated strain No 21/400 (R form). We ran the agglutination reaction with a live culutre (1 billion microbial cells) of strain No 543 and Schu, and variants thereof with the above-mentioned sera in dilutions of 1:5-1:2560. We demonstrated some differences in titers and nature of the agglutinate in the original strains and their erythromycin variants. The original Schu strain was agglutinated by serum to strain No 503 to a titer of 1:640 (stable agglutinate) and not by serum to strain No 21/400; the variant reacted with both sera in a titer of 1:80, agglutination by serum to strain No 21/400 being unstable. Both the original and variant of strain No 543 reacted in a titer of 1:320 with serum to strain No 503. The original strain did not react with serum to strain No 21/400, whereas the variant yielded a positive agglutination reaction (unstable) in a titer of 1:10.

These data are indicative of certain changes in antigenic properties of the strains in the direction of transformation from the S to the R form, which is related, as we know, to loss of Vi antigen. These changes were more marked in the Schu strain variant than No 543, in which they were only incipient.

Thus, analysis of the submitted data warrants the conclusion that acquisition of resistance to erythromycin by the strains is associated with a change in their morphological and cultural properties (change from S to R form), drastic attenuation of virulence and immunogenicity, and partial change in antigenic properties related to loss of surface Vi antigen. It should be noted that there was no parallelism of changes in antigenic and immunogenic properties. For example, strain No 543 lost its immunogenicity entirely but showed little change in antigenic properties, whereas the Schu variant showed more significant changes in antigenic properties than immunogenic ones. As indicated previously [2], the biochemical properties (attitude toward glycerin and citrulline) remained unchanged.

## Conclusion

Avirulent R variants were obtained by using erythromycin on the Schu strain (Nearctic race) and No 543 strain (Central Asian race). They differ from the S forms of the pathogen of tularemia in morphology of colonies, cultural and biological properties.

## PHOTO CAPTIONS

1. p 99. Mixture of culture of original and erythromycin variant of Schu strain (S and R forms); magnification 3×.

2. p 99. Mixture of cultures of original and erythromycin variant of strain No 543 (S and R forms); magnification 3×.

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# IMMUNIZATION WITH BIVALENT AND TRIVALENT MIXED VACCINES AGAINST PLAGUE, TULAREMIA AND ANTHRAX USING A JET INJECTOR. REPORT 1

Moscow ZHURNAL MIKROBIOLOGII, EPIDEMIOLOGII I IMMUNOBIOLOGII in Russian No 5, May 1974 (manuscript received 5 Feb 73) pp 59-64

[Article by V. G. Pilipenko, M. A. Miroshnichenko and N. A. Loktev, Scientific Research Plague Control Institute of the Caucasus and Transcaucasus, Stavropol]

[Text] For the last 10-15 years, the attention of many researchers had been drawn to the jet (needle-less) method of administering vaccines which, it is believed, simplified and accelerated considerably vaccination and renders this procedure virtually painless [7, 1, 2, 5, 6].

Various killed (monovalent vaccines, combined and polyvalent vaccines) and live vaccines were tested with the jet injector, in particular, there have been published reports on using the jet injector for inoculation of live monovalent vaccines against tularemia, plague and anthrax [9, 8, 3, 4, 5]. It was noted that an intensive inflammatory reaction with production of necrosis in the middle developed after administration of tularemia vaccine in the usual dilution to animals (guinea pigs, rabbits) [9]. Increased reactogenicity was observed when a jet injector was used to administer STI anthrax vaccine in the usual dosage [12]. These circumstances prompted the recommendation to use for injections tularemia vaccine diluted 20-40 times and anthrax vaccine in 5-10-fold dilutions. In such cases, a rather satisfactory immunological response was obtained, with moderate local postinoculation reactions.

Thus, in addition to satisfactory immunological efficacy of tularemia and anthrax vaccines demonstrated in experiments with the jet injector, there were reports of increased reactogenicity of these vaccines with the above method of administration. This shows that it is necessary to continue with investigations of the feasibility of administering live bacterial vaccines using a jet injector.

In view of the good prospects for using jet injectors, our objective was to investigate the possibility of using this method for immunization using live associated divaccines and trivaccines [divalent and trivalent vaccines] for immunization against plague, tularemia and anthrax, information about testing of which we could not find in the literature. In experiments on guinea pigs, we made a comparative determination of reactogenicity, safety and immunological efficacy of these live mixed vaccines and corresponding live monovaccines when administered with a jet injector.

In all, we conducted 7 experiments on 160 guinea pigs weighing 400-500 g. In the first experiment, we gave a mixture of plague and tularemia vaccines, in the second one a mixture of plague and anthrax vaccines, in the third a mixture of tularemia and anthrax, in the fourth a mixture of plague, tularemia and anthrax vaccines (there were 26 guinea pigs in each of the first 3 tests and 40 in the fourth). In the next experiments (5th, 6th, 7th), the animals were given monovalent plague, tularemia and anthrax vaccines, respectively (control).

The mixed vaccines were prepared from 2-day subcultures of vaccine strains of P. pestis EB and F. tularensis No 15 of Gayskiy (reconstituted). As the third component, we used standard dry STI anthrax vaccine (series No 123), produced at the Tbilisi Institute of Vaccines and Sera. The mixed vaccines, like the monovalent ones, were injected in a volume of 0.5 ml in the lateral shaved surface of the thigh using a Soviet BIP-4 jet injector at an effective pressure of 8 atm. In this volume of vaccine, there were 100 million plague, 10 million tularemia bacteria and 10 million live spores of STI vaccine, i.e., 20-30 times less than the usual doses of these vaccines when given separately by the epicutaneous method.

At the site of vaccination, all of the guinea pigs had noticeable infiltrates the size of a lentil, occasionally pale yellowish (lemon peel) in color with barely noticeable spot defect in the center (site of penetration of the jet). In some animals, blood seeped from this spot for the first min after injection.

Table 1. Local reaction of guinea pigs to mixed and monovalent vaccines

| Vaccine | Size of site of inflammation (mm) | | Necrosis | Duration of reaction (days) |
|---|---|---|---|---|
| | range of fluctuation | average | | |
| Mixture of plague and tularemia | 12×12—30×35 | 20×20 | + | 12—17 |
| Mixture of plague & anthrax | 15×15—30×40 | 26×30 | + | 15—17, less often 21 |
| Mixture of tularemia & anthrax | 12×12—35×37 | 22—24 | — | 14—19 |
| Mixture of 3 vaccines | 12×12—30×45 | 25×30 | + | 14—21 |
| Plague | 9×9—20×30 | 16×18 | — | 9—12 |
| Tularemia | 17×17—20×30 | 20×24 | + | 14—17 |
| Anthrax | 9×9—20×30 | 15—17 | — | 10—12 |

In guinea pigs immunized with a mixture of plague and tularemia vaccines, there were well-marked infiltrates and hyperemia of the skin at the injection site by the 2d-3d day (Table 1). By the 5th day, necrosis of the skin developed in the center of the infiltrated region. In most guinea pigs, the local reaction lasted 12-17 days, and in some animals it disappeared by the end of the 3d week. An equally strong local reaction, with formation of a necrotic area in the center of the focus of inflammation, developed in guinea pigs given a mixture of plague and anthrax vaccines (see Table 1). In the vast majority of animals the reaction disappeared by the 15th-17th day, less often the 21st. A moderate local reaction without marked necrosis of the skin was observed in animals inoculated with a mixture of tularemia and anthrax vaccines (see Table 1). The reaction usually regressed by the 14th, 17th and 19th days.

The most intensive reaction was observed in guinea pigs inoculated with a mixture of all three vaccines (see Table 1). In eight animals, the reaction was associated with formation of abscesses and extensive necrosis of the skin, which resembled the scabs of anthrax carbuncles in density, unevenness and dark color (see Figure, A [photo not reproduced]). The reactions regressed by the 12th-21st day, whereas complete recovery of tissue was observed at the end of the 4th week in some animals.

Thus, unlike inoculations by the cutaneous method with mixed bivalent and trivalent vaccines [10, 11], intracutaneous-subcutaneous administration using the BIP-4 jet injector for the same vaccines but 20-30 times less concentrated led to summation of reactogenic properties of their constituents and development of intensive local postvaccinal reactions.

In control guinea pigs inoculated with the jet injector using live monovalent vaccines, the local reactions were moderate (see Table 1 and Figure, Б, В, Г)

There was an 0.5-1° elevation of temperature in virtually all animals for 5-8 days after immunization. The animals were less active up to the 10th day, they did not take enough feed, lost an average of 24-25 g weight each and, in some cases, particularly in the second and fourth experiments, they lost 115-150 g. On subsequent days, the animals gained weight evenly, and by the end of the 40th day the weight of most guinea pigs returned to the initial level or increased somewhat.

We tested the serological response and allergic status on 12 guinea pigs from each experiment 40 days after immunization.

We found positive allergic reactions to percutaneous injection of tularin and anthraxin in 100% of the immunized animals (Table 2).

Blood serum of all animals immunized with bivalent and trivalent vaccines showed positive interaction with tularemia diagnosticum in the agglutination reaction, with mean titers of 1:148, 1:216 and 1:220, respectively; in animals given monovalent tularemia vaccine the titer was 1:406.

Serum from 75-100% of the animals showed a positive agglutination reaction with plague (erythrocyte) diagnosticum (PHAR [passive hemagglutination reaction]). The highest mean serum titer (1:218) was obtained in guinea pigs inoculated with monovalent plague vaccine and the lowest (1:72) with serum from animals given a mixture of plague and tularemia vaccines. The serum of animals inoculated with a mixture of plague and anthrax vaccine interacted positively with plague diagnosticum in higher dilutions--1:143 (Table 3).

Thus, all of the guinea pigs inoculated with mixed vaccines using a jet injector presented allergic alteration for tularemia and anthrax antigens to almost the same

Table 2.
Allergic change in guinea pigs 40 days after inoculation with mixed and monovalent vaccines using jet injector

| Vaccine | Experiment No | Allergen | Number of animals | Guinea pigs with positive reaction | Average reaction (in mm) |
|---|---|---|---|---|---|
| EV + LTV | 1 | Tularin | 12 | 12 | 12×12 |
| EV + STI | 2 | Anthraxin | 12 | 12 | 12×12 |
| | | Tularin | 12 | 12 | 13×14 |
| LTV + STI | 3 | Anthraxin | 12 | 12 | 12×12 |
| EV+LTV+STI | 4 | Tularin | 12 | 12 | 12×12 |
| | | Anthraxin | 12 | 12 | 9×9 |
| LTV | 6 | Tularin | 12 | 12 | 14×14 |
| STI | 7 | Anthraxin | 12 | 12 | 14×14 |

Figure 3.
Serological change in guinea pigs 40 days after immunization with mixed and monovalent vaccines

| Vaccine | Exper. No | Diagnosticum | Animals | | Mean titer |
|---|---|---|---|---|---|
| | | | total | positive reaction | |
| EV + LTV | 1 | Plague | 12 | 9 | 1:72 |
| | | Tularemia | 12 | 12 | 1:148 |
| EV + STI | 2 | Plague | 12 | 12 | 1:143 |
| LTV + STI | 3 | Tularemia | 12 | 12 | 1:216 |
| EV+LTV+ STI | 4 | Plague | 12 | 11 | 1:98 |
| | | Tularemia | 12 | 12 | 1:220 |
| EV | 5 | Plague | 12 | 12 | 1:218 |
| LTV | 6 | Tularemia | 12 | 12 | 1:406 |

Key for this and Tables 3 and 4:
EV) plague vaccine
LTV) live tularemia vaccine
STI) anthrax

extent as animals inoculated with monovalent tularemia and anthrax vaccines. All or the vast majority of animals presented antibodies to tularemia and plague antigens, and in all cases the reactions were distinct, being somewhat more marked to the tularemia vaccine used in mixture with other vaccines and separately. Serum titers referable to tularemia and plague antigens were higher in animals inoculated with monovalent vaccines than in those inoculated with mixed vaccines.

We assessed immunity to plague, tularemia and anthrax according to results of infecting the guinea pigs with virulent cultures of the corresponding pathogens 40-45 days after immunization. The animals immunized with mixed bivalent and trivalent vaccines were divided into two and three equal groups, respectively, then submitted to hypodermic infection with one of the pathogens. Guinea pigs immunized with monovalent vaccines and nonimmunized animals infected with the same pathogens served as controls.

We infected 12 animals from each experiment with 200 Dcl [?] of virulent strain No 461 of Y. pestis, 1000 Dcl of virulent culture of F. tularensis strain No 144/713 and 10 Dcl of anthrax vaccine No 2 of Tsenkovskiy. Among guinea pigs immunized with mixed and monovalent vaccines, 91-100% were found to be highly resistant to plague, 83-100% resistant to tularemia and 91-100% to anthrax (Table 4). Only in the third experiment did 1 out of 12 guinea pigs survive after immunization with anthrax vaccine. As shown by our subsequent tests with the Pchelka [Bee] jet injctor, the results of which will be published in our next report, such a low yield of animals immune to anthrax after administration of a mixture of tularemia and anthrax vaccine is not a chance phenomenon. Having recurred in other analogous experiments, it warrants reference with every justification to competition between these two antigens--

the adverse effect of tularemia vaccine on immunizing properties of anthrax vaccine, when administered together with a jet injector. However, addition of a third constituent, EV plague vaccine, to the mixture of the two abovementioned vaccines eliminated entirely the inhibitory effect of tularemia vaccine on immunological efficacy of STI vaccine.

Table 4.
Immunity of guinea pigs immunized with mixed and monovalent vaccines against plague, tularemia and anthrax

| Vaccine | Number of animals immunized against | | | | | |
|---|---|---|---|---|---|---|
| | plague | | tularemia | | anthrax | |
| | infected | survived | infected | survived | infected | survived |
| EV + LTV | 12 | 11 | 11 | 11 | — | — |
| EV + STI | 12 | 12 | — | — | 12 | 12 |
| LTV + STI | — | — | 12 | 12 | 12 | 1 |
| EV | 12 | 12 | 12 | 12 | 12 | 11 |
| EV | 12 | 12 | — | — | — | — |
| LTV | — | — | 12 | 10 | — | — |
| STI | — | — | — | — | 12 | 9 |
| Unimmunized guinea pigs | 10 | 0 | 10 | 0 | 10 | 0 |

Thus, animals immunized with mixed and, particularly trivalent vaccines using a jet injector showed as good an intensity of immunity as guinea pigs given monovalent vaccines. In all instances, development of resistance in the guinea pigs to the pathogens of plague, tularemia and anthrax was associated with distinct serological and allergic changes.

The pathomorphological and histological changes in the viscera of guinea pigs 60 days after administration of mixed vaccines by the jet injector method were essentially similar to the changes following use of the corresponding monovalent vaccines. They consisted of signs of moderate lymphoid and reticular hyperplasia, plasma cell reaction in lymph nodes, some hemosiderosis of the spleen, small proliferates of Kuppfer's cells in the liver, as well as insignificant perivascular lymphohistiocytic infiltration in the lungs. In only one case did the lungs show signs of interstitial pneumonia after immunization with a mixture of tularemia and anthrax vaccines. At the same time, it should be stressed that residual manifestations of nonsuppurative lymphadenitis and periadenitis were observed more often with use of a jet injector to give EV monovalent plague vaccine than to give other monovalent and polyvalent vaccines.

## Conclusions

1. When given with a BIP-4 jet injector, mixed bivalent and trivalent vaccines containing one-twentieth and one-thirtieth, respectively, of the usual doses for cutaneous inoculation of plague, tularemia and anthrax vaccines resulted in development of highly persistent resistance to plague, tularemia and anthrax in 90-100% of the guinea pigs; however, a significant part of the animals developed severe postvaccinal reactions at the site of injection of the mixed vaccines, with signs of necrosis, whereas guinea pigs immunized with the corresponding monovalent vaccines presented moderate local postimmunization reactions.

2. The severe reactions at the administration site were indicative of summation of reactogenic properties of the constituents of live mixed vaccine when given with a jet injector, which makes it imperative to continue studies for determination of optimum doses of constituents of mixed vaccine for administration with a jet injector.

## PHOTO CAPTION

p 61. Dynamics of local reaction of guinea pigs inoculated with mixed vaccine (A) and polyvalent--plague (Б), tularemia (В) and anthrax (Г)--vaccines. The numbers refer to postimmunization day of reaction

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# P ANTIGEN OF FRANCISELLA TULARENSIS

Moscow ZHURNAL MIKROBIOLOGII, EPIDEMIOLOGII I IMMUNOBIOLOGII in Russian No 7, Jul 74 (manuscript received 26 Feb 73) pp 52-56

[Article by V. V. Sukhar', Rostov-on-Don Plague Control Institute]

[Text] El'bert and Gayskiy established the existence of antigenic differences between virulent and avirulent strains of F. tularensis [9]. Olsuf'yev and Yemel'yanova [3] found that attenuation to an avirulent, nonimmunogenic state deprives the pathogen of tularemia of the so-called Vi antigen complex. However, it remained unclear whether the Vi complex is an individual antigen or corresponds to several antigens. We [4] discovered an individual antigen A, using the gel precipitation method, and demonstrated that it was wanting in avirulent cells [6], and it has been isolated in pure form [7]. In this report, we shall demonstrate that avirulent cells are wanting in yet another individual antigen, which was first discovered in an immunochemical study of the sline [mucus] fraction of F. tularensis and named P antigen.

In our study, we used two strains of F. tularensis--virulent No 128 (1 Dcl for white mice is 10 bacteria) and completely avirulent, nonimmunogenic No 21/400, as well as F. novicida (1 Dcl for white mice equals 10 bacteria). We recovered bacterial mass by desiccating with acetone 3-day cultures grown at 37° in glucose-cystine fish agar.

The fraction of mucous substance was obtained in the following manner: 122 g bacterial mass of strain No 128 was suspended in a 10-fold (weight/volume) amount of 0.85% NaCl solution (pH 7.0), heated, stirring occasionally, in a water bath to 57° and kept at this temperature for 1 h. The suspension was then placed in a beaker heated to 57° and centrifuged for 15 min at 30,000 G. The cellular sediment was discarded and, after cooling, we added to the centrifugate an aliquot of cold (-20°) acetone; the precipitate was separated form the liquid phase by centrifugation, dissolved at 57° in 250 mℓ 0.85% NaCl and placed in the refrigerator (1-2°) overnight, then centrifuged for 1 h at 30,000 G. The precipitated light bluish, semiclear mucous gel was dialyzed against distilled water and lyophilized.

We identified the antigen composition of the fraction using the reaction of double diffusion precipitation in agar (RDP) according to Ouchterlony [20] and a micromodification of this method--microprecipitation reaction according to

Kanchukh [1], using a multiwell plate [5] where the distance between wells is 3-5 mm.

Immunoelectrophoretic analysis of the fraction was made under the following conditions. The wells (4 mm in diameter) in a plate (120×90×2 mm) of 1% agar or agarose on Michaelis veronal buffer (pH 8.6, $\mu = 0.06$) were filled with a solution of the mucoid substance treated according to Sevag [23] to remove protein; we then added Sephadex G-25 to the well to create a continuous gel structure. After electrophoresis (I = 25 mA, potential gradient 8 V/cm, 1.5-3 h) the troughs 3 mm away from the wells were filled with immune serum.

We tested the antigenic properties of the fraction on chinchilla rabbits weighing 2.5-3 kg. The animals were given intravenous injections of 0.1--0.2--0.5--1--2--4--8 mg fraction at 6-day intervals. Before each injection, we took 1 mℓ blood from the marginal vein of the ear to determine the titer of serum agglutinins.

Agglutinating antisera to the fraction were obtained from rabbits given intravenous injections of 0.25--0.5--1--2 mg of the preparation at 6-day intervals. Antisera to live strain No 128 bacteria inactivated with 0.25% formalin were obtained by intravenous injection of 0.25--2--3--3 mg lyophilized cells at 1-week intervals.

A water-salt extract of dry bacteria was recovered by a method that included maceration with sand and extraction with 2.5% NaCl solution [4].

The yield of mucoid fraction constituted 2.54% of the weight of bacterial mass. In this fraction, we demonstrated 10.41% nitrogen by the method of Fels and Veatch [13], 3.4% phosphorus by the method of Fiske and Subbarow [14], 4.59% protein by the method of Lowry et al. [17], 16.8% carbohydrates according to Dubois et al. [12], 0.13% nucleic acids according to Spirin [8], but no DNA using the method of Dische. Maximum amount of reducing substances (10.43%) when scaled to glucose was assayed according to Hagedorn and Jensen [16] after 6 h hydrolysis with 2 N HCl at 100°, amino sugars (10.4%) according to Cessi and Piliego [10] after 7 h hydrolysis with 0.2 N HCl at 100°, N-acetylated amino asugars (3.86%) according to Reissig et al. [21] after 1 h hydrolysis with 0.2 N HCl at 100°. After hydrolysis with 6 N HCl, there was precipitation of a significant sediment of substance soluble in chloroform and mixture of chloroform with methanol (2:1), which was indicative of presence of a lipid component in the preparation.

Using the reaction of diffusion precipitation with tularemia equine and rabbit antisera to live strain No 128 bacteria, within the fraction we demonstrated A antigen and 2 antigens in common in tularemia strains No 128 and 21/400, as well as F. novicida. Antiserum to formalin-treated strain No 128 bacteria and antifraction serum demonstrated only common antigens, but not A antigen.

Figure 1 illustrates the results of testing the antigenic properties of the mucoid fraction; it had good agglutinogenic properties: 0.8-3.8 mg of the substance injected during the first 3-5 injections elicited a significant elevation of agglutinin titers. However, upon subsequent injections of large doses (4-8 mg) they declined.

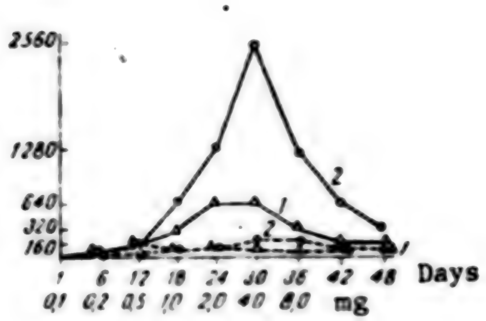

Figure 1.
Dynamics of agglutinin titers in blood serum of rabbits No 1 (1) and 2 (2) in response to intravenous injection of mucoid fraction of strain No 128 bacteria

Solid lines--agglutinins to virulent strain No 128; dash lines--to virulent strain No 21/400.
X-axis, dosage given; y-axis, reciprocals of agglutinin titers

Proceeding from the data obtained in the last experiment, we prepared and tested the following system for recovering agglutinating antifraction serum: rabbits were given intravenous injections of 0.25, 0.5 1 and 2 mg fraction at 6-day intervals. The sera thus obtained presented titers of 1:640-1:2560 in the agglutination reaction with virulent F. tularensis and 1/32-1/64 these levels with avirulent tularemia bacteria. In the RDP with water-salt extracts (1-15 mg) of both tularemia strains and F. novicida, the antifraction sera caused formation of only 1-2 lines of precipitation common to the tested strains, but did not precipitate A antigen, which was indicative of absence of A antibodies.

Agglutination by antifraction sera of tularemia bacteria wanting in A antibodies should mean that the sera contain agglutinins to an unknown surface antigen (agglutinogen) differing from antigen A. We named the new agglutinogen P antigen. The low agglutination titers of antifraction sera to strain No 21/400 warranted the belief that P antigen is absent in avirulent tularemia cells. We obtained direct proof of this in the experiment where 2 antifraction sera with agglutination titers of 1:640 to strain No 128 and 1:80 to strain No 21/400 lost the agglutinins to the avirulent strain, but retained them completely for the virulent strain after absorption of 100 mg bacterial mass of strain No 21/400 (2 h at 37° and 24 h at 4°).

We were unable to demonstrate P antigen in the RDP according to Ouchterlony: 4-16 mg water-salt extract or 20 mg bacterial mass of strain No 128 did not cause appearance of visible precipitation lines with the antifraction serum (P serum) absorbed by the avirulent strain, whereas only 1-2 lines in common with the virulent strain were formed with unsorbed serum, and they could not belong to P antigen. Conversely, the microprecipitation reaction with P serum diluted in such a way that its titer in the agglutination reaction was 1:160-1:640 and the mucoid fraction (1:100-1:1000) or water-salt extract (1:10-1:100) of bacteria of virulent strain No 128 consistently demonstrated P antigen in the form of a line, which was usually narrower than the A antigen line (Figure 2 [photo not reproduced]).

Rabbit antisera to live strain No 128 bacteria absrobed by the bacterial mass of strain No 21/400, or equine tularemia sera contained A and P antibodies (AP sera). We were able to demonstrate A and P antigens immediately with use thereof in the microprecipitation reaction.

Immunoelectrophoresis of the mucoid fraction (1:250-1:2500) using rabbit AP serum (titer after dilution 1:200-1:400) also demonstrated P antigen: on agar electrophoregrams there was a line of A antigen closer to the cathode; between it and the well was a line of P antigen (Figure 3 [photo not reproduced]). Immunoelectrophoresis in agarose gel showed that A and P antigens remained where they had been applied, which was indicative of their electrophoretic neutrality.

All of the tested virulent (Nos 128, 1657, 1658) and vaccine (Nos 9, 10, 11, No 15 of Gayskiy--reconstituted and American No 38) strains revealed P antigen by the method of bacterial agglutination. They were agglutinated to a titer or one-half by P serum. The minimally immunogenic vaccine strain No 15 Gayskiy (nonreconstituted) was an exception, and it agglutinated to one-quarter titer. Evidently the extremely low P antigen content of this strain (about 1/500th of the amount in strain No 15 Gayskiy--reconstituted--and 1/1000th of that in virulent strains) is the cause of its poor agglutinability.

Brucella (Br. abortus 554, Br. melitensis 16M, Br. suis 1330, the single brucella diagnosticum of series No 19, Odessa Institute of Virology and Epidemiology) and F. novicida were not agglutinated by P serum, which was indicative of species-specificity of P antigen.

In addition to the extracts already mentioned, P antigen was demonstrated in trichloroacetic (according to Boivin), water-ether [19] and water-acetone (1:1) extracts. A method of isolating it in pure form has not yet been developed, but distribution is already known according to some methods of separating the extract [7]. According to these data, P antigen is eluated in the external part of the column when filtered through Sephadex G-100, migrates to the cathode upon electrophoresis in a starch block (pH 8.75), is not sorbed on a column with the acetate form of DEAE-Sephadex, but is sorbed and partially separates from A antigen on a column with the borate form of this anion-exchange resin.

The properties of this antigen have been studied. It was found to be resistant at 20° to trichloroacetic acid, formamide, 0.25% formalin and detergents (1-5% sodium desoxycholate and sodium dodecylsulfate), boiling for 2 h and autoclaving (110°, 40 min). When boiled for 30 min in 0.1 M NaOH or 0.2 M $Na_2CO_3$, antigens A and P were destroyed, whereas only P antigen was destroyed when boiled for 1 h in 0.1 M acetic acid, and this could be used as a differential test for quantitative assays of A antigen.

In the fraction obtained by chromatography on DEAE [diethylaminoethyl] Sephadex, which contained P antigen, carbohydrates were demonstrated without admixture of proteins and nucleic acids [7]. These data, as well as resistance to denaturing factors, warrant the assumption that P antigen is a carbohydrate.

In order to produce a P line in the microprecipitation reaction, 300 times more water-salt or water-ether extract is required than for formation of the A line. This difficulty in demonstration of P antigen is apparently the reason why, in the experiments of Saslaw et al. [22], some sera with high agglutination titers from people immunized with Foshey killed tularemia vaccine did not cause formation of precipitation lines, whereas in the experiments of Nutter [18] agglutinating rabbit antisera to extracts of tularemia bacteria caused only a precipitation band reduced to the form of a mist near the antigen well.

It was previously reported [7] that only live, rather than killed bacteria and extracts of both, elicited A antibody production in rabbits. In contrast, considerable titers of P antibodies were recovered with administration of formalin-treated bacteria or the mucoid fraction. It can be assumed that expressly P antibodies determine the agglutination titers of antisera to killed bacteria and extracts, whereas in antisera to live cells apparently A antibodies are also agglutinins.

The antigenic receptors that are lost with attenuation of F. tularensis have been designated heretofore as Vi antigenic substances, Vi antigen complex or Vi antigen [2, 3]. It was assumed that this complex is a membrane that invests the cell [3]. In view of the specificity for immunogenic (virulent and vaccinal) strains, antigens A and P could be viewed as the first individual antigens of the Vi complex to be identified. Thus, it can be concluded that the Vi antigen complex is heterogeneous, and the hypothesis can be expounded that the surface of the pathogen of tularemia constitutes a complex mosaic pattern made of up A and P antigens, as well as two agglutinogens in common with Brucella, the existence of which was mentioned already by Francis and Evans [15].

We have yet to determine the significance of A and P antigens in the mechanism of virulence and immunogenicity. For this purpose, it will be necessary to recover them in pure form and select $A^+P^-$ and $A^-P^+$ variants of F. tularensis.

## Conclusions

1. An antigen, named P antigen, specific to virulent and vaccine strains of F. tularensis, has been discovered.

2. P antigen is a thermostable, species-specific agglutinogen.

3. Monoreceptor P serum has been recovered for quantitative assay of P antigen using serological methods.

## PHOTO CAPTIONS

2. p 54. Demonstration of P antigen by microprecipitation reaction
*а*: 1) rabbit P serum (1:320*): 2) rabbit AP serum (1:640*); 3) equine AP serum series 38-2 (1:320*); 4) equine AP serum series 38-2 (1:1280); 5) equine AP serum series No 68-2 (1:1280*); 6) mucoid fraction** (1:500)
*б*: 1) 0.85% NaCl; 2) equine AP serum series No 38-2 (1:1280); 3) rabbit P serum (1:320*); 4) rabbit AP serum (1:320*); 5) rabbit AP serum (1:1280); 6) mucoid fraction** (1:500)
*в*: 1) water-salt extract of strain No 128 (1:40); 2) mucoid fraction** (1:500); 3) mucoid fraction** (1:250); 4) 0.85% NaCl; 5) water-salt extract of strain No 21/400 (1:20); 6) rabbit AP serum (1:640)

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*Agglutation titers after diluting sera.

**Here and in Figure 3, the fraction recovered from strain No 128 and treated according to Sevag.

3. p 55 Demonstration of P antigen by immunoelectrophoresis
*а*, *в*) rabbit AP serum (1:320); *б*) normal rabbit serum; 1) mucoid fraction** (1:250); 2) mucoid fraction** (1:2500); 3) mucoid fraction** (1:20,000)

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# EVALUATION OF VACCINE STRAINS OF F. TULARENSIS IN EXPERIMENTS ON RABBITS

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[Article by L. V. Sirotyuk, State Institute for Standardization and Control of Medical Biologicals imeni Tarasevich, Moscow]

[Text] Immunological quality of vaccine strains is of paramount importance to high efficacy of live tularemia vaccine. Immunogenicity of vaccine strains of F. tularensis is determined on guinea pigs, which are highly sensitive and highly susceptible to tularemia (infective and lethal dosage for them consists of a few bacteria). Man is also highly susceptible to tularemia and can be easily infected, but because of low sensitivity the disease usually ends with recovery. The rabbit is just as susceptible to tularemia, and a few bacteria are sufficient to infect it, but even 10 billion bacterial cells does not constitute an unconditionally lethal dose for it [3]. For this reason, we were interested in the rabbit as, perhaps, a closer model to man in order to evaluate vaccine strains of F. tularensis.

Since rabbits do not die when infected with highly virulent strains of the Palearctic variant of F. tularensis, we decided not to study immunity by the infection method, and we conducted a comparative study of antigenicity and "taking" of several vaccine strains of F. tularensis in the collection of the Institute imeni Tarasevich. We used genetically related variants of tularemia vaccine strain Gayskiy No 15, which differed in immunogenicity for guinea pigs: highly immunogenic variant No 15 NIIEG, the $ED_{50}$ of which for guinea pigs numbers a few bacteria, moderately immunogenic variant No 15 v/IIa with $ED_{50}$ constituting thousands of bacteria and the mildly immunogenic variant No 15/38, the $ED_{50}$ of which for guinea pigs is up to millions of bacteria.

In our tests, we used 48-h second-generation cultures grown on solid yolk medium at 37°. We inoculated chinchilla rabbits weighing 2-2.5 kg with cultures suspended in isotonic solution of sodium chloride to a density of 2 billion bacteria per milliliter, according to the optical standard of cloudiness for F. tularensis. The inoculation was administered by the scarification method: we applied 2 drops of suspension (one inoculation dose for man) to a depilated skin area on the animals side and, using a smallpox vaccination quill, made parallel scratches 0.8-1.0 cm long through the drops, after which we rubbed the suspension in with the flat side of the quill for 30 s, then allowed it to dry in the air for 5-10 min. The reaction was considered positive when hyperemia and an infiltrate at least 0.5 cm in cross section appeared at the site of scarification on the 5th postvaccinal day.

We took blood from the marginal vein of the ear from all rabbits, before inoculation and every 7 days for 1 month. We ran the hemagglutination reaction and passive hemagglutination reaction (PHA), being governed by the techniques described in the technical documentation for tularemia diagnosticum and erythrocytic tularemia diagnosticum. As antigen, we used a suspension of second-generation culture of vaccine strain No 15 v/IIs, which was grown on McCoy medium at 37°. The suspension contained 5 billion bacterial cells per milliliter isotonic solution of sodium chloride, pH 7.0. Rabbit sera were inactivated prior to the PHA by heating at 56° for 30 min. Erythrocytes sensitized with tularemia antigen were obtained from the Institute of Epidemiology and Microbiology imeni Gamaleya.

The highest elevation of agglutinin titer was observed in most rabbits on the 14th day, then the quantity of agglutinins started to diminish and held at an average level (Table 1). More intensive antibody production was observed when strains No 15 NIIEG [Scientific Research Institute of Epidemiology and Hygiene] and 15 v/IIs were used for vaccination. When the rabbits were immunized with strain No 15/38, most of them failed to produce any agglutinins at all. Maximum accumulation of hemagglutinins was also observed on the 14th day in most rabbits, after which they began to diminish and held at an average level (see Table 1) for the duration of the observation period (28 days). The three strains differed drastically in capacity to elicit hemagglutinin production: NIIEG No 15 presented the greatest antigenicity (hemagglutinin titer exceeded 1:8000 on the 14th day in most rabbits), strain No 15 v/IIs elicited moderate hemagglutinin production (titers did not exceed 1:2000) and strain No 15/38 had a mild effect (in most rabbits hemagglutinin titers did not exceed 1:160).

Table 1. Dynamics of agglutinins and hemagglutinins in blood of rabbits immunized with variants of Gayskiy No 15 strain

| Antibodies | Strain No | Mean titers (log) | | | | | Number of rabbits |
|---|---|---|---|---|---|---|---|
| | | before imm. | after immunization (day) | | | | |
| | | | 7th | 14th | 21st | 28th | |
| Agglutinins | 15v/IIs | 0 | 2,21 | 2,31 | 1,98 | 1,79 | 9 |
| | 15 NIIEG | 0 | 1,95 | 2,31 | 2,19 | 2,19 | 9 |
| | 15/38 | 0 | 0 | 1,34 | 1,32 | 1,0 | 9 |
| Hemagglutinins | 15v/IIs | 0 | 2,28 | 3,26 | 3,04 | 2,94 | 9 |
| | 15 NIIEG | 0 | 3,53 | 3,81 | 3,61 | 3,47 | 9 |
| | 15/38 | 0 | 1,83 | 3,02 | 2,80 | 2,30 | 9 |

Thus, only immunogenically active variants of Gayskiy vaccine strain No 15 v/IIs and 15 NIIEG elicited agglutinin production. Mashkov and Belkina [2] made analogous findings: when rabbits were given Gayskiy strain No 15 vaccine, agglutinins appeared in their blood in a titer of up to 1:640, whereas with administration of an avirulent tularemia strain no agglutinin production was observed. Production of hemagglutinins in the blood of immunized rabbits occurred concurrently, and the maximum levels of both were observed at the same time, on the 14th day. The capacity to elicit hemagglutinin production was related to immunogenicity of the vaccine strains: the more immunogenic the strain, the higher the hemagglutinin titer in blood of rabbits immunized with it. Hemagglutinin titers exceeded significantly the agglutinin titers in the same rabbits, and they were often present in blood in the complete absence of agglutinins. On the basis of the foregoing, it can be considered that use of PHA as a test for antigenicity with sera of rabbits immunized cutaneously could be useful in the study of vaccine strains.

In addition to antibody production, we examined acceptance of cultures of different vaccine strains of F. tularensis in immunized rabbits, since this is one of the criteria for evaluating strains on guinea pigs [1]. In humans, a close link was also noted between acceptance and quality of the strain [5].

In the tests of acceptance ["take"], in addition to the strains used to test antigenisity, we used the mildly immunogenic variant of Gayskiy strain No 15/Kb, the $ED_{50}$ of which for guinea pigs constituted millions of bacteria, mildly immune strain No 33 NIIEG with $ED_{50}$ of hundreds of thousands of bacteria and highly immunogenic strains of NIIEG Nos 10 and 53, the $ED_{50}$ of which constitute less than 10 bacterial cells [7].

Table 2.
Take in rabbits of vaccine strains of F. tularensis

| Strain No | Rabbits, out of 12, with positive reaction to dose of | | | |
|---|---|---|---|---|
| | 50 000 | 500 000 | 5 000 000 | 50 000 000 |
| 15v/IIs | 7 | 11 | 12 | 12 |
| 15 NIIEG | 9 | 11 | 12 | 12 |
| 15/38 | 0 | 2 | 4 | 9 |
| 15/Kb | 1 | 3 | 5 | 8 |
| 10 NIIEG | 11 | 12 | 12 | 12 |
| 33 NIIEG | 8 | 10 | 12 | 12 |
| 53 NIIEG | 10 | 12 | 12 | 12 |

Experiments were conducted in three variants. In the first variant, we tested 4 doses at a time on each rabbit, from 50,000 to 50 million bacteria of the same strain (4 rabbits per strain). We use dose to refer to the quantity of bacteria in two drops of suspension applied to the rabbit's skin for immunization. We conducted three tests, in which all of the strains except the mildly immunogenic ones, No 15/38 and 15/Kb elicited similar results (Table 2). Thus, with use of even mildly immunogenic strain No 15 Kb one rabbit presented a distinct take up to a dosage of 50,000 bacteria, whereas with administration of highly immunogenic strain No 15 NIIEG one rabbit presented a positive reaction only up to a dosage of 500,000 bacteria. Apparently, such a system of titration for acceptance by one animal is unsuitable for determining the quality of a strain.

In the second variant of experiments, we immunized one rabbit simultaneously with all strains, but in one dilution which, if the strain had immunogenic properties, elicited positive immunization reactions in most rabbits. Five rabbits were immunized cutaneously with all strains in a dosage of 50,000 bacteria. The vaccinal reactions were the same in all animals: positive with strains No 15v/IIs and NIIEG No 10, 15 and 53; negative with strains No 15/38, 15/Kb and 33 NIIEG. Thus, this method is apparently more promising for comparative studies of acceptance of vaccine strains.

In the third variant, we tested three No 15 strains differing in immunogenicity--No 15v/IIs, 15 NIIEG and 15/38. Each strain was tested on the same rabbit in the same inoculation dosage, which constituted 200 million bacteria according to the opacity standard. We conducted three experiments, with three rabbits per strain in each test. On the 5th day, all rabbits immunized with No 15v/IIs and 15 NIIEG presented positive immunization reactions in all three experiments; however, with use of strain No 15/38, a take was observed only in the first experiment in one out of the three rabbits, whereas no immunization reaction was present in the rest of the cases.

Consequently, the set-up of the first and third variants of experiments made it possible to differentiate the acceptance only of strains that had changes which were advanced in the direction of saprophytization of the type in strains 15/38 and 15/Kb. When all strains were applied in the same dilution simultaneously to one rabbit (second variant), we were already able to single out, according to immunogenicity, even the mildly immunigenic strains such as No 33 NIIEG. We were unable to demonstrate the initial stages of decline of immunogenicity in the 15v/IIs type strain according to immunization reactions of the rabbit. It should be noted that we obtained a response in rabbits that is similar to the type of response of man, since tests of humans also failed to demonstrate, on the basis of take, any appreciable differences between highly immunogenic strain No 15 NIIEG and moderately immunogenic strain No 15v/IIs [4]. Thus, although the results of rabbit studies referable to the take test are not suitable for evaluation of immunogenicity of commercial vaccine strains, they can still be used in experimental work. Studies of rabbits are also quite promising for evaluation of commercial strains with regard to testing antigenicity of vaccine strains, since hemagglutinin production was consistent with degree of immunogenicity.

## Conclusions

1. There was a correlation between antigenicity of tularemia strains for rabbits when cultures were applied on the skin and their immunogenicity in tests with guinea pigs.

2. A study of hemagglutinin titers in epicutaneously immunized rabbits made it possible to demonstrate an initial decline of immunogenicity of vaccine strains of F. tularensis, whereas agglutinin titers in similarly immunized rabbits reflected only advanced changes in the strain, in the direction of saprophytization.

3. Vaccinal skin reactions in the rabbits made it possible to demonstrate only a significant decline of immunogenicity of vaccine strains of F. tularensis.

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# STUDY OF ENDEMIC INFECTIONS IN LITHUANIAN SSR. REPORT 3: TULAREMIA INFECTION AMONG RURAL POPULATION ACCORDING TO RESULTS OF SEROLOGICAL AND ALLERGOLOGICAL TESTS

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[Article* by L. I. Moteyunas, Republic Sanitary and Epidemiological Station, Vilnius]

[Text] In the period of keeping records in the USSR, tularemia has been found in all administrative regions and republics of the European part of our country, with the exception of Lithuanian SSR [7]. In order to determine whether there are also endemic sites for tularemia in the last mentioned republic, initially a study was made of such environmental elements as landform, natural biocenoses and parasitocenoses. On the basis of landform features, it was established [3] that there are conditions in Lithuanian SSR for existence of sites of the meadow-field, forest and backwater-swamp types. The fauna referable to small wild mammals is represented in Lithuania by 17 species of highly susceptible and highly sensitive animals [6] with respect to tularemia (group I according to Olsuf'yev and Dunayeva), which are capable of maintaining a continuous epizootic process in nature. In this republic, there are 18 species of bloodsucking arthropods (6 species of ticks, 4 of gadflies [or horseflies] and 8 mosquito species), which are potential vectors of F. tularensis, and ticks are also capable of maintaining persistence of endemic sites. On the basis of evaluation of the gathered data, from the standpoint of medical geography and landform epidemiology, the hypothesis was expounded that there are endemic tularemia sites in Lithuania [3]. It was checked by means of allergological and serological testing of the rural population in 1969-1972, including amateur hunters who are exposed to a higher risk of tularemia infection.

In our survey, we took into consideration the age, time of residence in the screened region, occupation, how long the subject had been engaged in hunting and how the hare carcasses were handled. Allergic tests wer performed by percutaneous injection of 0.1 m tularin. The reaction was checked after 24-48 h. It was considered positive if there was an infiltration (induration) of the skin at the site of tularin injection at least 5 mm in diameter. In view of the strict specificity of the reaction to tularin and the fact that preventive inoculations against tularemia had never been given to the inhabitants in LiSSR, all cases of positive allergic reactions to tularin were evaluated as retrospectively detected cases of prior infection. On the basis of the results of the epidemiological survey, all individuals who reacted positively to tularin were divided into three groups: the first consisted of individuals infected by F. tularensis within the territory of Lithuanian SSR, the second those who very probably were infected in this republic, but there was no absolute certainty of this; the third group consisted of individuals who had been infected (reliably and probably) or immunized outside Linthuania.

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*ZHURNAL MIKROBIOLOGII, EPIDEMIOLOGII I IMMUNOBIOLOGII, 1973, No 5, p 83 and No 11, p 130.

We took blood for the agglutination reaction (AR) just prior to the allergic skin test. We used the volumetric AR with tularemia diagnosticum, which is considred highly sensitive and strictly specific [1, 5, 8]. We recorded reactions with a titer of at least 1:20. Considering the antigenic similarity of pathogens of tularemia and brucellosis, in all positive AR cases we also ran a test with brucella diagnosticum. We recorded the AR and allergic skin test separately, without taking into consideration the results of the other test.

We performed the allergy test on 73,534 people ranging in age from 18 to 69 years; 5372 of them were hunters (all men) living in 25 administrative regions of the republic and the other 68.162 lived in rural areas in 18 (out of 44) regions. Of those screened, 84.8% had lived in the area of the screening for over 10 years, 79.9% of the hunters had hunted for over 5 years and 68.4% always hunted in the same administrative district.

Since Lithuanian SSR is a territory that consists of several separate ecological and faunal regions (EFR), the obtained data were analyzed not only all together, but for the different regions [9].

According to the allergy test, the highest percentage of positive reactions was found among hunters in the southeastern EFR (Table 1), which is in first place among other regions with regard to lakes and forests.

Table 1. Results of allergy test with tularin made on hunters

| EFR | Number of hunters | | Incidence of positive reactions | | | |
|---|---|---|---|---|---|---|
| | under observat.* | tested | epidemiological groups | | | total |
| Northwestern | 2576 | 1868 (72,5) | 50 | 12 | 13 | 75 (4,0±0,4) |
| Central Lithuanian | 2827 | 2003 (70,9) | 30 | 17 | 8 | 55 (2,8±0,4) |
| Southwestern | 892 | 725 (81.3) | 23 | 4 | 5 | 32 (4,4±0,8) |
| Southeastern | 1368 | 776 (56,7) | 27 | 11 | 10 | 48 (6,2±0,9) |
| Totals | 7663 | 5372 (70,1) | 130 (2,4±0,2) | 44 (0,8±0,1) | 36 (0,7±0,1) | 210 (3,9±0,3) |

*In the tested administrative regions of the indicated EFR.
Note: Percentages are given in parentheses.

Hunters with positive reactions (including those referable to 1st and 2d epidemiological groups) were found in all 25 regions surveyed; however, the percentage thereof fluctuated over a wide range (from 0.8±0.5 to 7.8±2.4). The largest number of individuals with positive tularin reactions (8.2±1.2%) was found among hunters who are agricultural machine operators, who are exposed to the additional risk of tularemia infection during their work (dismantling stacks, loading, transporting and unloading fodder, sugar and feed beets, etc.). They repeatedly mentioned seeing epizootics in the stacks (according to epidemiological screening charts). In second place according to incidence of positive reactions were foresters (4.6±1.0%).

The highest percentage of positive reactions among hunters was noted in the 20-29 and 30-39 year age groups (4.6±1.0 and 5.6±0.5, respectively). After 40 years, there was gradual decline in number of individuals with positive tests with each passing decade (4.1±0.4 and 1.7±0.4%, respectively), and it was the lowest (1.3±0.4%) in the age group of 60 or more years old. An analogous tendency was found when we analyzed data with consideration of duration of hunting activities: the highest incidence of positive tularin reactions was found among hunters who had been hunting for up to 10 years (4.5±0.6 and 5.2±0.6% for the first and second 5-year periods, respectively). This percentage gradually dropped in subsequent periods.

However, not only and not so much the actual hunting process as the process of primary handling of hare carcasses influence infection of hunters (Table 2).

Table 2. Allergic reaction of hunters to intracutaneous injection of tularemia as related to frequency of stripping hare carcasses

| Indicator | Total | Did strip carcasses | | |
|---|---|---|---|---|
| | | always | sometimes | did not |
| Number tested: absolute | 5180 | 4840 | 152 | 188 |
| % | 100 | 93,4 | 3,0 | 3,6 |
| Positive reactions: abs. | 199 | 193 | 4 | 2 |
| % | 3,8±0,3 | 4,0±0,3 | 2,6±1,3 | 1,0±0,7 |

Among the rest of the inhabitants, positive reactions to tularin constituted 0.68±0.04% among women (out of 36,398) and 2.09±0.08% (out of 31,764 men), i.e., 3.1 times more. Of the 909 people with positive tularin reaction, 369 (40.6%) were referred to the first epidemiological group, 211 (23.2%) to the second and 329 (36.2%) to the third. The lowest incidence of positive reactions (0.43±0.07%) was found among individuals up to 19 years of age. In other age groups, it was higher: 1.4±0.2 at 20-29 years, 1.9±0.3 at 30-39 years, 1.4±0.3 at 40-49 years, 1.0±0.1 at 50-59 years and 0.6±0.1% at over 60 years of age.

The distribution of individuals with positive tularin reactions in different occupational groups was as follows: 4.2±0.4% among foresters, 3.3±0.3% among land reclamation workers, 2.5±0.2% among agricultural machine operators, 1.6±0.1% among blue- and white-collar workers, 0.8±0.05% among agricultural workers and 0.55±0.07% among "others" (students, housewives, retirees and others).

In a scheduled serological screening of 3953 foresters and land reclamation workers, a positive AR was found in 4.5±0.3% (3.9±0.4 and 5.0±0.5%, respectively). In an analogous scheduled screening of 5629 foresters and land reclamation workers of the same regions using the allergy test, positive results were found in 3.7±0.2% of the cases. Consequently, one can pick up a somewhat larger number of immune individuals with the AR test than with the allergy test alone ($t = 1.9$). The results of the allergy and AR test did not coincide in a considerable number of individuals. For this reason, if we consider all those in whom either of these tests was positive, the actual immune element in the relevant groups with regard to tularemia would be larger than established solely by the allergy test.

A comparative study of infection of hunters and other population groups was made on the basis of data for 13 regions (in the rest of the regions, such a study was made only for hunters, whereas other strata of the population were not studied, or vice versa). The group of hunters consisted of 2792 people and the group of other rural population, identical in age and sex to the hunters, consisted of 20,476 people. As shown by a comparison for only the first epidmeiological group, which is the most correct, there were 3.6 times more hunters with positive tularin reactions than among the rest of the inhabitants (2.9±0.3 and 0.8±0.06%, respectively).

We evaluated the sensitivity of both methods and suitability of retrospective detection of individuals who had suffered from tularemia infection by means of simultaneous screening of 1573 hunters living in 8 rural regions, using the allergological and serological (AR) tests. The results revealed that when the selected population group (1573 people) was screened by either of the above tests the percentage of positive findings constituted 4.1-4.3±0.4, whereas with concurrent use of both tests it reached 6.3±0.6.

Since agglutinins and allergic sensitivity, which are highly sensitive and specific in individuals with a history of tularemia, persist for decades [2, 5, 8], it was necessary to determine whether the endemic sites had retained activity up to recent years, or whether the demonstrated immune stratum constituted merely a trace of prior (long ago) activity. For this purpose, we screened 3159 school children 7 to 16 years of age from 23 schools in 2 regions of the republic. Our choice of schools was governed by data from the allergy screening of the adult population (in areas of maximum accumulation of positive reactions). We found 20 children (0.6±0.1%) from 7 schools who reacted positively to intracutaneous injection of tularin, including 8 children with positive reactions out of 198 tested in one school. Of the 20 children with positive reactions, 9 were up to 10 years of age. According to the epidemiological survey, all 20 children could have been infected with tularemia only within the borders of the repbulic, and 18 of them only within the borders of their administrative regions. Serological testing of 14 school children with positive tularin tests revealed that AR was positive in titers of 1:20 and 1:40 in only 2 of them. Other authors have also observed that the results of allergy and serological tests on children did not coincide [2]. The submitted data indicate that, in addition to endemic sites for arboviral infections [4, 9], there are also tularemia sites in Lithuanian SSR.

Conclusions

1. In Lithuanian SSR, there are endemic tularemia sites, in which hares are the reservoirs of infection in nature and source of human infection.

2. For the population of Lithuania there are other routes of tularemia infection, in addition to a route such as hunting.

3. The most complete retrospective detection of individuals infected with tularemia is achieved by concurrent use of allergological and serological tests.

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# TULAREMIA IN THE ARCTIC

Moscow ZHURNAL MIKROBIOLOGII, EPIDEMIOLOGII I IMMUNOBIOLOGII in Russian No 12, Dec 74 (manuscript received 25 Dec 73) pp 17-19

[Article by L. I. Nekrasova, Arkhangelsk Medical Institute]

[Text] The region least explored with regard to tularemia is the tundra zone, which occupies vast expanses of the Soviet Union. There are sparse data in the literature concerning tularemia in the Arctic, and they deal with detection of this infection primarily in the Extreme North of European USSR. Thus, in 1953-55, 17 cases of tularemia in man, confirmed by serological and allergic skin tests were diagnosed in the tundra zone of Kola Peninsula. The outbreaks were preceded by an unidentified epizootic among lemmings and other rodents; these animals were not examined bacteriologically at that time [5]. Endemic tularemia sites were also discovered beyond the North Pole circle and in the Nenetsk National Okrug of Arkhangelsk Oblast [4]. As for the extreme northern Asian part of the USSR, a tularemia site was found on the lakes of the forest-tundra in the suburbs of Norilsk [1, 3]; an extensive zoological and parasitological survey of the fauna and epidemiological screening of the public proved the existence of endemic tularemia sites on the banks of the Ob River inlet, at the latitude of the Arctic circle [2]. There is no information concerning tularemia in the rest of the territory of the Arctic region in the Asian part of the USSR.

For this reason, we tried to determine the distribution of tularemia and intensity of epidemic manifestation of endemic sites of infection in the extreme northern part of Siberia and the Far East, by means of serological screening of inhabitants, who are permanent residents of this region and who were not immunized against tularemia. We excluded from this survey individuals who were serving in the Soviet Army. We determined the natural immune element among aborigenes (Nenets, Dolgan, Nganasan, Yakut, Chukchi, Eskimo and others) in the summertime, in different areas: Chukotsk National Okrug in 1970, Taymyr National Okrug in 1971, Yamalo-Nenetsk National Okrug in 1972, Bulunskiy, Allaykhovskiy and Nizhnekolymskiy Rayons of Yakutsk ASSR. All of the populated centers surveyed were situated in the tundra zone and only two villages (Kolymskoye and Andryushkino) in the forest-tundra zone.

For the epidemiological survey, we used the agglutination test with highly sensitive diagnosticum from a strain of F. tularensis of the Neoarctic subspecies (the diagnosticum was obtained from the Institute of Epidemiology and Microbiology imeni Gamaleya, USSR Academy of Medical Sciences). We ran the agglutination reaction with 1:20 serum dilution, and a very distinct (+++) reaction was considered positive.

Results of serological testing for tularemia of indigenous population in the extreme northern part of Siberia and the Far East

| Testing site | Number of people tested | Number of people tested who showed positive agglutination test result (≥1:20) | |
|---|---|---|---|
| | | absolute | %±m |
| Yamalo-Nenetsk National Okrug: | | | |
| Yar-Sale | 103 | 21 | 20.3±4 |
| Taymyr National Okrug: | | | |
| Novorybnoye | 138 | 27 | 18.9±3 |
| Novaya | 44 | 4 | 9±4 |
| Yakutsk ASSR: | | | |
| Nayba | 160 | 32 | 20±3 |
| Chokurdakh | 102 | 16 | 15.6±3 |
| Kotenko | 90 | 13 | 14.4±4 |
| Oyotung | 87 | 13 | 14.9±4 |
| Kolymskoye | 53 | 6 | 11.3±4 |
| Andryushkino | 104 | 16 | 15.4±3 |
| Chukotsk National Okrug: | | | |
| Uelen | 81 | 13 | 16±4 |
| Nunyamo | 83 | 7 | 8.4±3 |
| Yanranay | 22 | 2 | 9±6 |
| Ryrkaypiy | 48 | 5 | 10.4±4 |

We tested 1115 people 10 to 70 years of age in 13 populated centers. We found positive agglutination reactions in a serum dilution of 1:20-1:160 (mainly in dilution of 1:20-1:40) in inhabitants of all regions screened (see Table). In the youngest age group (10-14 years), positive reactions were obtained in 4% (out of 113 tested). The percentage of people immune to tularemia was highest among those over 20 years of age, and it ranged from 9 to 23 in the different localities. There was a history of protracted sore throat, lymphadenitis, fever, i.e., diseases that were suspicious for tularemia, in only 24 subjects, whose blood showed specific agglutinins.

We also used the Wright test in all cases of positive serum reactions to tularemia. In two cases, we obtained a positive result in titers that did not exceed the ones with tularemia antigen (1:20-1:40).

Aside from the existence of a naturally immune stratum among the indigenous inhabitants, the cases of tularemia among individuals who came to these regions from other places for temporary work also served as confirmation of the presence of active endemic sites for tularemia. Thus, there were two cases of tularemia in scientists who had worked in an expedition to Taymyr Peninsula, the region of Pyasina River, near Tareya (73° north latitude) in July and August 1967. The infection occurred against a background of numerous lemmings, which is the background species of tundra rodents.

Here is a brief excerpt from a case history:

> Patient P., 38 years old, participated in expedition to Taymyr starting on 10 July. She had been sick since 15 August. For 5 days there was general malaise, increased fatigability. On 22 August, her temperature rose to 39.2°, she suffered from severe chills, headache and pain in the right inguinal region. On 23 August, the patient noticed enlargement of lymph nodes in the right inguinal region and swelling with hyperemia of the skin, 3 cm in diameter, at the site of a bite, in the middle third of the right lower leg. Her temperature was 38° for 1 week. She was treated with tetracycline. Her well-being improved, so that she was able to continue working in the field. On 2 September, her condition worsened again: temperature rose again to 38°, pain reappeared and lymph nodes were enlarged in the right inguinal region. After returning to Leningrad on 6 September she was hospitalized in the surgical department of the hospital of the Academy of Sciences, from which she was transferred to the Infectious Hospital imeni Botkin with suspicion of tularemia. The diagnosis of ulcerative-bubonic form of tularemia was made on the basis of her history, clinical findings and results of immunological test (on 20 October positive agglutination reaction in a dilution of 1:320; a 1×1.5 cm papule appeared at the site of tularin injection 48 h later, which was surrounded by a hyperemic zone 4 cm in diameter). She responded well to streptomycin.
>
> Another participant of this expedition became sick on 30 July. After a few days he noticed a bubo in the inguinal region. He took tetracycline on his own. Infection by the bubonic form of tularemia with localization of the bubo in the neck region had already been observed in 1965 in one scientist during expeditionary work in this same region of Taymyr Peninsula. In that case, the diagnosis of tularemia was confirmed serologically and positive result of skin test with tularin upon retrospective examination in 1967.

## Conclusions

1. A serological screening of a sample of 1115 indigenous residents, who had not been immunized against tularemia, made it possible to retrospectively ascertain prior tularemia infection, which is indicative of the existence of endemic tularemia sites in the Arctic region of Asian USSR.

2. The northernmost endemic tularemia site was found on Taymyr Peninsula (73° north latitude), where three cases of tularemia were recorded among members of an expedition.

3. The obtained data validate the desirability of immunizing individuals for whom there is an occupational risk of tularemia infection in the Extreme North.

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# SEROLOGICAL EXAMINATION OF PREDATORY BIRD PELLETS IN THE VICINITY OF THE IMAM-BABA STATION IN TURKMEN SSR

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[Article by V. M. Neronov, B. P. Dobrokhotov and I. S. Meshcheryakova, Institute of Epidemiology and Microbiology imeni Gamaleya, USSR Academy of Medical Sciences]

[Text] In 1962, a base was organized in the vicinity of the Imam-Baba Station, which is situated in the central course of Murgaba River, by the Institute of Epidemiology and Microbiology imeni Gamaleya, USSR Academy of Medical Sciences, in order to investigate the many-year changes in main elements of a site of zoonotic cutaneous leishmaniasis, and for the last 2 years tularemia and plague.

In 1951, Khodukin [11], who examined the history of tularemia in Asia, arrived at the conclusion that the minor epidemic outbreak described by Voskresenskiy, which was recorded in 1884 in the garrison at Merv, resembled tularemia in all of its features. Subsequently, Strelyayeva et al. [8], who screened the local inhabitants (using the tularin skin test) and serum from cattle, goats and sheep from different parts of Turkmenia, also concluded that there was a possibility of tularemia sites in the Murgaba valley.

An outbreak of pneumonic plague was observed in late 1912 in the vicinity of Merv. In the fall of the same year, many deaths were observed among rodents, but it was not possible to confirm bacteriologically that a plague epizootic had occurred [10]. In 1950 and 1957, a plague epizootic was discovered among gerbils far to the south of the base (in the vicinity of Kushka, village of Chemen-i-Bit and Pobeda Sovkhoz), but the isolated strains presented attenuated virulence [3]. A similar type of Y. pestis strain had also been isolated in the vicinity of Iolotan, north of the base [2]. On the basis of analysis of environmental conditions and distinctions of the isolated strains, Akiyev [1] assumed that it is unlikely for the plague enzootic to persist in Southeastern Kara-Kumy, whereas the elevations of Badkhyz and Karabil to the south could merely be a zone to which Y. pestis is carried from other endemic plague sites.

As had already been repeatedly demonstrated [4-6, 12], serological testing of predatory bird pellets is a rather sensitive method for detection of epizootics of both tularemia and plague. On the basis of these studies, we collected pellets in the valley and desert-sand regions in the vicinity of our base [7]. The first batch of pellets (112) was collected from 7 to 15 February and the second (172 units) from 28 April to 11 May 1973. It should be noted that, in the winter of 1973, there was a considerable increase in number of great gerbils in most parts of the base after several years of a low population thereof (the preceding peak was reported in 1964-1965, and in 1966-1967 there was a depression). In 1973, there was also an increase in number of red-tailed Libyan jirds and house mice. In turn, the abundance of feed and the features of the winter of 1972-1973 caused an increased concentration of predatory birds (kestrels [Falco species], long-legged buzzards, kites, steppe eagles, vultures and others) in the vicinity of Imam-Baba: we had occasion to observe up to 9-10 birds per field of vision in February over the third terrace [bench] of Murgaba. But, by the end of April, most of them had migrated or flown to the north, and the usual pattern became established in these sites with prevalence of the common and lesser kestrels [9] and rare sightings of other, larger species (long-legged buzzard in the sand and kite near the village). In February 1973, we collected pellets under the power-line poles in the hilly-ridged sands about 1-1.5 km from the left main bank of Murgaba. We failed to collect fresh pellets there 2.5 months later, although this was expressly the location of the highest density of great gerbil burrows (see Table) and the poles were very convenient as resting places for birds of prey--on the hills, next to the road and cattle-driving areas. In addition to these poles, some pellets were collected under telegraph poles on the second terrace on the left bank of Murgaba, under a few turanga trees in the tugays [vegetation-covered bottomland] on the right bank of the Murgaba, on the hills, under bushes and under the two triangulation marks on the third terrace and flattened-wavy sands on the right bank of the Murgaba. But, in general there were few pellets, 1-2 at some points and up to 10 in rare cases. All were relatively fresh; old and decomposed pellets that could be attributed to prior years were virtually absent.

The gathered pellets were distributed as follows with regard to weight: first batch (February 1973, total of 112)--under 2 g for 13 pellets, 2-5 g for 73, over 5 g for 26; second batch (April and May 1973, total of 172)--under 2 g 49 pellets, 2-5 g 59 pellets and over 5 g 64. The smaller ones were apparently referable to the kestrels, and were gathered chiefly in the tugays; average-sized ones were referable to kites and buzzards, large ones to spotted and steppe eagles, and others.

All of the pellets were tested by the antibody neutralization reaction, using standard methods [4, 5], for detection of tularemia and plague antigens. No tularemia antigen was demonstrable in the pellets. In four cases, plague antigen was found in the first batch of pellets: No 13 (from the hilly and ridged sands, residues of the great gerbil, pellet weight 7 g, titer 1:24), No 67 (third terrace of the Murgaba, residue of the great gerbil, pellet weight 7.4 g, titer 1:96), No 77 (third terrace, residues of great gerbil and insects, pellet weight 15.3 g, titer 1:48) and No 92 (second terrace on the left bank of the Murgaba, fur and bone residue, species unknown, pellet weight 6.3 g, titer 1:12). The last dilution of tested material causing complete arrest of passive hemagglutination scaled to dry pellet weight was taken as the reaction titer. We used plague erythrocytic diagnosticum (dry) prepared by the Alma-Ata Plague-Control Institute as antigen to run these reactions. No plague antigen was found in the second batch of pellets. It should be noted that they were gathered beyond the limits of the area where pellets had been gathered in February, and there were virtually no fresh pellets at the old collection sites. Typically enough, plague antigen was demonstrated in the largest pellets of the first batch, and three of them contained residue of the great gerbil, which is the principal carrier in endemic plague sites of Central Asia.

Results of recording density of great gerbil burrows, number of predatory birds and distribution of their pellets under power line poles (hilly-ridged sands on the left bank of the Murgaba River)

| Place and time of recording and gathering pellets | Distance traveled (km) | Number of active great gerbil burrows[1] | Number of predatory birds encountered | Number of power line poles examined | Number of poles where pellets were gathered (quantity of pellets in paren.) |
|---|---|---|---|---|---|
| Between Bolshevik village and Imam-Baba station, 28 Apr 73 (1100-1315 hours) | 5 | 13 | 1 kestrel | 20 | 12(32) |
| Between Beriket and Imam-Baba 29 Apr 73 (1000-1245 hours) | 3.25 | 44 | 0 | 13 | 4(12)[3] |
| North of road, Egri well Imam-Baba station, 11 May 73 (1000-1200 hours) | 2.75 | 13 | 3 kestrels,<br>2 black vultures[2] | 11 | 4(18) |
| Total during 7-h excursion | 11 | 70 or 1.6/ha | 4 kestrels,<br>2 vultures | 44 | 20(62) |

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[1] Three recorders walked along the power lines, width of record tape 40 m.
[2] The carcass of a long-legged buzzard was found under one of the poles.
[3] On this segment of the route, pellets were collected on 12 Feb 73.

## Conclusions

1. Examination fo 284 pellets of predatory birds, collected in the vicinity of the Imam-Baba station in February and April-May 1973 using the antibody neutralization reaction failed to demonstrate tularemia antigen; plague antigen was demonstrated in titers of 1:12, 1:24, 1:48 and 1:96 in 4 pellets, in 3 of which there was residue from great gerbils.

2. In order to give a definitive answer to the question of whether there are endemic plague sites in this territory, as well as describe them, further investigations are needed.

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# POSSIBILITY OF PREDICTING PLASMOCYTE REACTION OF MICE TO SOLUBLE ANTIGEN

Moscow ZHURNAL MIKROBIOLOGII, EPIDEMIOLOGII I IMMUNOBIOLOGII in Russian No 3, Mar 75 (manuscript received 28 Mar 74) pp 104-112

[Article by M. I. Levi, N. N. Sakayan, M. M. Livshits, I. S. Meshcheryakova and I. V. Rodionova, Moscow Municipal Disinfection Station]

[Text] Zdrodovskiy [3] proposed the plasmocyte reaction as one of the indicators of cellular immunity. It was subsequently shown that accumulation of cells of the plasmocyte class in a regional lymphoid organ is a function of the antigenic stimulus, which determines the quantitative characteristics of the plasmocyte reaction [2, 7, 20]. The increment in plasma cells was proportionate to the square of the logarithm of administered dose of antigen, as well as modulus of transition from antigenic function to accumulation of plasma cells (a), and inversely proportionate to the specific rate of antigen elimination from the injection site (κ). Since there was no appreciable increase in incidence of mitosis in the lymphoid system after administration of soluble antigen, in accordance with data in the literature [14, 15, 17, 22] it was concluded that the plasma cell reaction to soluble antigen was largely attributable to an influx of plasma cell precursors in the regional lymphoid organ from other lymphoid structures [9]. Moreover, it was found useful to record the intensity of the plasma cell reaction when calculating precursors of antibody-producing cells, since it was necessary to add to the precursors in the regional lymphoid organ before administration of antigen the precursor cells that were found there as a result of influx of mobile cells from other lymphoid formations.

We undertook this study in order to determine the concrete value of the modulus of change from antigen function to plasma cells (a). In previous studies [7, 9, 20], modulus a was determined on the basis of overall increment of cells of the plasmocyte class in a regional lymphoid organ (spleen) and dynamics of antigen concentration at the site of administration (abdominal cavity). However, since the spleen cannot, strictly speaking, be considered a regional lymphoid organ for the abdominal cavity [19], we considered here the concentration of soluble antigen in blood, for which the spleen is, of course, a regional lymphoid organ.

We used two soluble antigens in our experiments: capsular antigen (fraction I) of P. pestis [18] and Boivin's complete antigen of F. tularensis [12, 13]. The capsular antigen was recovered from acetone-dried bacterial mass of vaccine strains EV and Kl (4, 16]. Complete F. tularensis antigen was recovered in dry form from standard strain 503/830 [12, 13]. After assaying protein according to Lowry and serological activity, solutions of these antigens were injected intraperitoneally to CBA mice in a volume of 0.25 mℓ, then we dissected 5 animals from each group given each dose, daily for 6-10 days (the dosage was usually reduced gradually to one-fourth). The method of examining the animals was described previously [9]. We used the antibody neutralization reaction (ANR) with stable antigenic diagnosticum and passive hemagglutination reaction (PHAR) with stable antibody diagnosticum [5, 11] by the micromethod (of Takacs) to determine antigen concentration in the entire volume of blood (mean serum volume was considered to be 3.5% of average mouse weight), after which we counted the average antigen molecules per nuclear cell in the spleen. We proceeded from the fact that the molecular weight of P. pestis antigen is 150,000 [1, 9, 10] and that of F. tularensis complete antigen 11,000,000. One minimum neutralizing dose of capsular antigen determined in the ANR was considered to equal 0.001 µg protein of "pure," i.e., without significant impurities, capsular antigen, whereas 1 hemagglutinating unit (HU) of the same antigen, as determined in the PHAR, was estimated as 0.0001 µg protein of "pure" capsular antigen [9]. We used

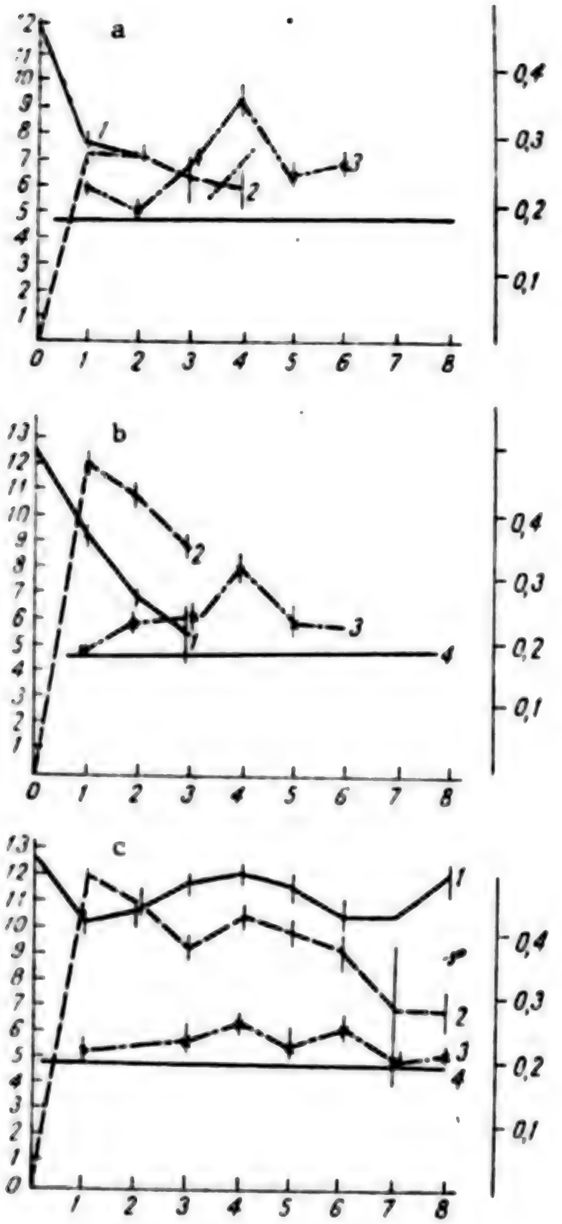

Figure 1.
Dynamics of concentration of soluble antigen and plasma cell reaction →

a) capsular antigen (experiment No 20, $\ln \frac{D_0}{N} = 12.0$; PHAR)
b) capsular antigen (experiment No 14, $\ln \frac{D_0}{N} = 12.6$; ANR)
c) complete antigen (experiment No 9, $\ln \frac{D_0}{N} = 12.7$; PHAR)

1) antigen concentration in abdominal cavity
2) in blood
3) probability of plasma cells in spleen of immunized animals $PC_0$)
4) nonimmunized animals ($PC_k$)

X-axis, time (days) after giving antigen; y-axis, left--natural logarithm of concentration of antigen molecules/nuclear spleen cell; right--probability of plasma cells among nuclears in spleen the following estimates for F. tularensis complete antigen: 1 minimum neutralizing unit equals 0.1 μg dry antigen, 1 HU = 0.01 μg dry complete antigen. The erythrocyte diagnosticum of about the same sensitivity were matched over the entire period of our tests.

We ground the spleen of each mouse in a homogenizer; a suspension was prepared in Hanks solution, after which we counted nuclear cells in a Goryayev chamber, as well as total plasmocyte class cells (mature and immature plasma cells, plasmoblasts and blasts) under an MBI-6 phase-contrast microscope at magnification of 90 × 7 × 2.5 in 300-400 lymphoid cells encountered at random [7, 20].

As an example, we describe the dynamics of concentration of antigen in the abdominal cavity and blood, as well as concentration of plasma cells in several experiments only at the times when we succeeded in demonstrating it with the serological reactions (Figure 1). It is easy to see that the dynamics of the complete antigen differed considerably from the dynamics of capsular antigen.

Thus, the mathematical expression of modulus a acquires the following appearance:

$$\text{Modulus } \underline{a} = \frac{\int_t^{t+2} (PC_0 - PC_k)\,\Delta t}{\int_0^t \ln \frac{D_i}{N}\,\Delta t} \approx \frac{\sum_t^{t+2} (PC_0 - PC_k)\,\Delta t}{\sum_0^t \ln \frac{D_i}{N}\,\Delta t}, \qquad (1)$$

where a is the modulus of change from antigenic function to accumulation of plasma cells; $\Delta t$ is the time interval, which was 1 day in all of our experiments; $D_i$ is the amount of antigen in molecules over the entire blood volume at time $i$; N is the number of nuclear cells in the spleen; $PC_0$ is the probability of finding plasma cells among nuclears of the spleen at time $i$ in immunized animals; $PC_k$ is the probability of finding plasma cells in nonimmunized animals

$$\left[\sum_t^{t+2} PC_k\,\Delta t = (t+2)\,PC_k\right],$$

$t$ is the time of recording demonstrable amounts of antigen in blood (days).

The scales of values were submitted previously [9].

It must be noted that most of the intraperitoneally injected soluble antigen was demonstrable in blood within 1-2 h, for which reason we equated the amount of antigen in blood at the moment of injection with the amount of antigen given ($D_0$). The second distinction of equation (1) is that the plasmocyte reaction was estimated at the time when antigen was demonstrated in blood plus 2 days.

This restriction was used in order to keep track of differentiation in plasma cells of the mobile cells that were recruited to the spleen from other lymphoid structures under the influence of antigen. Moreover, the peak plasmocyte reaction was usually observed 2 days after a maximum concentration of antigen in blood was reached (Table 1).

It was logical to assume that the greater the value characterizing the background ($PC_k$), the greater the plasmocyte reaction:

$$\sum_{0}^{t+2} (PC_0 - PC_k)$$

and, consequently, modulus $\underline{a}$. For this reason, we introduced a new concept, the plasmocyte index $PC_i = \overline{a}/PC_k$. Incidentally, in a mathematical model [9] modulus $\underline{a}$ is encountered in expressly such a combination; moreover, unlike modulus $\underline{a}$, $PC_i$ is a dimensionless parameter. The value of $PC_k$ is most probably related to the animals' average weight (Figure 2). Another factor that influences the plasmocyte reaction is the purity of the capsular antigen preparation:

$$I = \left(ln\frac{d}{q}\right)^2 + 1,$$

where $q$ is estimation of minimum neutralizing dose or hemagglutinating unit, as given above, and $d$ is the weight characteristic of a specific antigen series. On the basis of the foregoing, we proposed that dimensionless parameter A be used as a constant:

$$\text{Parameter}\quad A = \frac{a}{PC_k} \cdot \frac{1}{\left[\left(ln\frac{d}{q}\right)^2 + 1\right]}. \qquad (2)$$

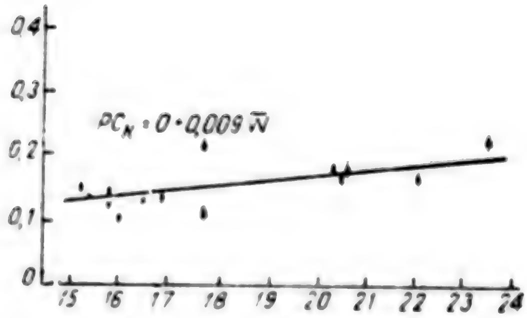

Figure 2.

Mean weight of CBA mice ($\overline{W}$) as a function of probability of finding plasma cells in spleen of nonimmunized animals ($PC_k$)

X-axis, mean mouse weight ($\overline{W}$)(g) in experiment; y-axis, probability of finding plasma cells in spleen of nonimmunized animals ($PC_k$). Arithmetic mean $PC_k$ and error of means, as well as line of regression (regression equation $PC_k = 0 + 0.009\ \overline{W}$)

Table 1. Values of modulus a in different experiments

| Exper. No | Agent used for immunization | Mean mouse wt. (g) | $PC_h$ | Sero-logical test | Antigen dose $\left(\ln\frac{D_t}{N}\right)$ | Obtained modulus $a \times 10^{-3}$ |
|---|---|---|---|---|---|---|
| 4 | Fraction I P. pestis K1 | 16,1 | 0,107 | ANR | 14,5; 13,0; 11,8 | 3,3; 2,7; 2,1 |
| | | | | PHAR | 14,1; 12,7; 11,3 | 3,6; 3,0; 2,6 |
| 10 | | 23,6 | 0,234 | ANR | 11,9; 10,5 | 4,8; 2,3 |
| | | | | PHAR | 13,5; 12,1 | 5,3; 3,3 |
| 14 | | 20,6 | 0,180 | ANR | 14,0; 12,6 | 8,5; 7,0 |
| | | | | PHAR | 12,6; 11,2 | 10,3; 7,3 |
| 20 | Fraction I P. pestis K1 + Fraction I P. pestis EV | 20,6 | 0,187 | ANR | 12,3; 10,9 | 11,0; 8,0 |
| | | | | PHAR | 12,0; 10,6 | 12,7; 10,8 |
| | | | | ANR | 12,2; 10,8 | 7,7; 8,0 |
| | | | | PHAR | 12,0; 10,6 | 9,5; 11,2 |
| 8 | Fraction I P. pestis EV | 17,0 | 0,162 | PHAR | 15,3; 13,9; 12,5 | 7,1; 4,6; 4,4 |
| 16 | | 20,4 | 0,190 | ANR | 15,6; 15,8; 15,6 | 3.8; 7,1; 5,9 |
| | | | | PHAR | 15,4; 15,4; 15,4 | 3,7; 7,1; 4,7 |
| 9 | Complete antigen F. tularensis | 20,8 | 0,190 | ANR | 13,4; 12,0; 10,6 | 9,6; 4,3; 2,4 |
| | | | | PHAR | 14,1; 12,7; 11,3 | 7,0; 4,5; 3,2 |
| 6 | | 16,6 | 0,141 | ANR | 12,8; 11,4; 10,0 | 5,9; 11,3; 9,3 |
| | | | | PHAR | 13,0; 11,6; 10,2 | 5,8; 6,8; 6,2 |

On the basis of the values of parameter A in different experiments (Table 2), we calculated the arithmetic means of modulus a and parameter A (Table 3), and both were compared according to coefficient of variation. For fraction 1 of P. pestis, the mean values of modulus a and parameter A had substantially different coefficients of variation when using the PHAR, but not ANR, which could be attributed to the longer demonstration of soluble antigen in blood and, for this reason, more accurate determination of the value of this parameter. Since we only had one series of complete antigen of F. tularensis at our disposal, the values listed in the table could merely serve to predict the plasmocyte reaction but not to compare variability thereof.

As it applies to consideration of concentration of soluble antigen in blood, the previously proposed [9] equation for the plasmocyte reaction can be written down in the following manner:

$$\frac{\sum_{t}^{t+2} PC_a \Delta t}{\sum_{t}^{t+2} PC_h \Delta t} = 1 + \frac{a}{PC_h} \cdot \frac{\sum_{0}^{t} \ln \frac{D_t}{N} \Delta t}{t+2}. \qquad (3)$$

Using equation (3) and mean values of parameter A for the entire set of experiments, we calculated the dimensionless variable in the left part of the equality, after which we compared the estimated value for each specific experiment to the one found experimentally, and we also calculated deviation of estimated value from experimental one (see Table 2). We found that the estimates did not deviate very significantly from experimental values.

Table 2. Determination of parameter A from data of different experiments and check for possibility of predicting plasmocyte reaction

| Exper. No | Preparation used for immuniz. | Amount of protein or complete antigen in dose given (μg) | Serol. test | Parameter $A \times 10^{-3}$ | $\frac{\sum_{t}^{t+2} PC_e \Delta t}{\sum_{t}^{t+2} PC_k \Delta t}$ |  | Deviation of estimate from experim. values (%) |
|---|---|---|---|---|---|---|---|
|  |  |  |  |  | estim. | exper. |  |
| 4 | Fraction I P. pestis K1 | 512 | ANR | 4,7 | 1,321* | 1,329* | 0,6*** |
|  |  |  | PHAR | 3,8 | 1,340 | 1,329 | —0,8 |
|  |  | 128 | ANR | 4,0 | 1,266 | 1,329 | 4,7 |
|  |  |  | PHAR | 3,3 | 1,342 | 1,329 | —1,0 |
|  |  | 32 | ANR | 3,1 | 1,266 | 1,167 | —8,5 |
|  |  |  | PHAR | 2,8 | 1,233 | 1,167 | —5,7 |
| 10 |  | 250 | ANR | 1,7 | 1,404 | 1,273 | —10,3 |
|  |  |  | PHAR | 5,2 | 1,133 | 1,298 | 12,7 |
|  |  | 62 | ANR | 0,8 | 1,361 | 1,174 | —15,9 |
|  |  |  | PHAR | 3,3 | 1,141 | 1,204 | 5,2 |
| 11 |  | 260 | ANR | 9,0 | 1,241 | 1,448 | 14,2 |
|  |  |  | PHAR | 4,5 | 1,411 | 1,448 | 2,5 |
|  |  | 65 | ANR | 7,2 | 1,226 | 1,310 | 6,4 |
|  |  |  | PHAR | 3,2 | 1,420 | 1,310 | 8,4 |
| 20 | Fraction I P. pestis K-1 + fraction I P. pestis EV | 62 | ANR | 6,4 | 1,305 | 1,453 | 10,2 |
|  |  |  | PHAR | 6,1 | 1,278 | 1,453 | 12,0 |
|  |  | 16 | ANR | 4,3 | 1,287 | 1,137 | 13,2 |
|  |  |  | PHAR | 4,9 | 1,272 | 1,218 | —4,4 |
|  |  | 64 | ANR | 4,1 | 1,314 | 1,303 | 0,8 |
|  |  |  | PHAR | 4,5 | 1,320 | 1,387 | 4,8 |
|  |  | 16 | ANR | 4,3 | 1,287 | 1,137 | 13,2 |
|  |  |  | PHAR | 4,9 | 1,272 | 1,218 | —[illegible] |
| 8 | Fraction I P. pestis EV | 420 | PHAR | 3,5 | 1,503 | 1,538 | 2,3 |
|  |  | 105 | PHAR | 2,3 | 1,453 | 1,538 | 5,5 |
|  |  | 26 | PHAR | 2,2 | 1,422 | 1,538 | 7,5 |
| 9 | Complete antigen F. tularensis | 400 | ANR | 50,5 | 1,342 | 1,383 | 3,1 |
|  |  |  | PHAR | 36,8 | 1,340 | 1,357 | 1,3 |
|  |  | 100 | ANR | 22,6 | 1,347 | 1,172 | —13,0 |
|  |  |  | PHAR | 23,8 | 1,321 | 1,224 | —7,9 |
|  |  | 25 | ANR | 12,6 | 1,282 | 1,079 | —15,8 |
|  |  |  | PHAR | 16,8 | 1,274 | 1,132 | —12,5 |
| 6 |  | 800 | ANR | 41,8 | 1,429 | 1,394 | —2,4 |
|  |  |  | PHAR | 41,1 | 1,337 | 1,394 | 4,3 |
|  |  | 200 | ANR | 80,1 | 1,342 | 1,604 | 19,5 |
|  |  |  | PHAR | 48,2 | 1,274 | 1,376 | 8,0 |
|  |  | 40 | ANR | 66,0 | 1,269 | 1,390 | 9,5 |
|  |  |  | PHAR | 44,0 | 1,249 | 1,313 | 5,1 |

*We used the mean parameter A (see Table 3); probability of plasma cells in nonimmunized animals was calculated with regression equation $PC_k = 0 + 0.009 \overline{W}$ (Figure 2).

**Values of $\bar{w}$, $\ln N$, $\sum_{0}^{t} \ln \frac{D_t}{N} \Delta t = \frac{\sum_{t}^{t+2} PC_e \Delta t}{\sum_{t}^{t+2} PC_k \Delta t}$ were found in the experiments.

***Deviation = $\frac{\text{experimental value - estimated value}}{\text{experimental value}} \times 100$.

Table 3. Mean values of modulus a and parameter A, and variability thereof

| Antigen | Compared signs | Serol. test | Number of tests (n) | Means (t) | Arithmet. mean (×10⁻³) | Coefficient of variation (%) |
|---|---|---|---|---|---|---|
| Fraction I P. pestis | Modulus a | ANR | 14 | 3,3 | 5,9±0,73 | 46,4 |
| | | PHAR | 17 | 4,0 | 6,5±0,79 | 54,5 |
| | Paramet. A | ANR | 11 | 3,3 | 4,5±0,72 | 53,3 |
| | | PHAR | 14 | 4,0 | 3,9±0,30 | 29,2 |
| Complete antigen F. tularensis | Modulus a | ANR | 6 | 2,5 | 7,1±0,14 | 48,0 |
| | | PHAR | 6 | 6,2 | 5,6±0,60 | 26,2 |
| | Paramet. A | ANR | 6 | 2,5 | 45,6±10,44 | 56,1 |
| | | PHAR | 6 | 6,2 | 35,1±4,98 | 31,7 |

Finally, we used the determined mean values of parameter A to predict accumulation of cells of the plasmocyte class in a special experiment, in which mice were given intraperitoneal injections of a mixture of different doses of P. pestis fraction I and F. tularensis complete antigen. We dissected 5 animals given each dose daily, for 8 days, in order to assay concentrations of both antigens and count plasmocyte elements. According to the previously established pattern for the variant of giving two different antigens [8, 21], we calculated accumulation of plasmocyte elements using the following equation:

$$\left[\frac{\sum\limits_{1}^{t+2} PC_e \Delta t}{\sum\limits_{1}^{t+2} PC_k \Delta t}\right]^{CA+FI} = 1 + \left[\frac{a}{PC_k} \cdot \frac{\sum\limits_{0}^{t} \ln \frac{D_t}{N} \Delta t}{t+2}\right]^{CA} + \left[\frac{a}{PC_k} \cdot \frac{\sum\limits_{0}^{t} \ln \frac{D_t}{N} \Delta t}{t+2}\right]^{FI}. \qquad (4)$$

The designations in this equation are referable to overall data for complete antigen (CA) or fraction I (FI), or for their combined effect (CA+FI). Since $PC_k$ was determined in the experiment (and it was found to be virtually identical to the estimate), we compared the estimates to experimentally found values under more rigid conditions. However, our results indicated that there is a very real possibility of predicting the level of the plasmocyte reaction, and the demonstrated deviations of estimates from experimental values were not so considerable (Table 4).

Thus, the proposed equations, which describe accumulation of cells of the plasmocyte class in the mouse spleen as a function of dynamics of concentration of soluble antigen in blood, enable us to predict the level of the plasmocyte reaction. We must stress the need to consider the dynamics of antigen concentration in blood. However, there are direct indications to the effect that dynamics of antigen at the injection site are a concrete function of administered dose [6, 9]. Most probably, the dynamics of antigen in blood are also a function of given dose of antigen, which experiences the influence of certain other factors (accumulation of antibodies in blood, elimination of antigen from the organism, etc.).

Table 4. Accumulation of plasmocyte class cells in spleen of mice given a mixture of P. pestis capsular antigen and F. tularensis complete antigen

| Dosage of antigen mixture* | Preparation used for immunization | Serol. test | $\ln\frac{D_0}{N}$ | $t$ | $\sum_{0}^{t} \ln\frac{D}{N} \Delta t$ | $\sum_{1}^{t+2} PC_{a\,\Delta t}^{CA+FI}$ | $\frac{a}{PC_k} \times 10^{-3}$** | $\frac{\sum_{1}^{t+2} PC_{a\,\Delta t}^{CA+FI}}{\sum_{1}^{t+2} PC_{k}\Delta t}$ |  | Deviation of estim. from experimental value (%)**** |
|---|---|---|---|---|---|---|---|---|---|---|
|  |  |  |  |  |  |  |  | experimental*** | estim. |  |
| Large | Fraction I<br>P. pestis | ANR | 12,1 | 5 | 64,1 | 1,660 | 13,5 | 1,7449 | 1,5660 | 24,0 |
|  |  | PHAR | 10,5 | 5 | 55,1 | 1,660 | 32,4 | 1,7449 | 1,6165 | 17,2 |
|  | Compl.antigen<br>F. tularensis | ANR | 15,1 | 6 | 77,2 | 1,844 | 45,6 | 1,7449 | 1,5660 | 24,0 |
|  |  | PHAR | 15,1 | 6 | 82,7 | 1,844 | 35,1 | 1,7449 | 1,6165 | 17,2 |
| Medium | Fraction I<br>P. pestis | ANR | 10,7 | 3 | 40,3 | 0,982 | 13,5 | 1,4670 | 1,5101 | −9,2 |
|  |  | PHAR | 9,1 | 3 | 33,8 | 0,982 | 32,4 | 1,4670 | 1,5174 | −5,3 |
|  | Compl.antigen<br>F. tularensis | ANR | 13,8 | 4 | 53,1 | 1,180 | 45,6 | 1,4670 | 1,5101 | −9,2 |
|  |  | PHAR | 13,7 | 4 | 51,3 | 1,180 | 35,1 | 1,4670 | 1,5174 | −5,3 |
| Small | Fraction I<br>P. pestis | ANR | 9,3 | 1 | 16,0 | 0,597 | 13,5 | 1,4927 | 1,4277 | 13,2 |
|  |  | PHAR | 7,7 | 3 | 27,2 | 1,005 | 32,4 | 1,5045 | 1,4536 | 10,1 |
|  | Compl.antigen<br>F. tularensis | ANR | 12,4 | 2 | 31,2 | 0,806 | 45,6 | 1,4927 | 1,4277 | 13,2 |
|  |  | PHAR | 12,3 | 4 | 47,3 | 1,213 | 35,1 | 1,5045 | 1,4536 | 10,1 |

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*Mean mouse weight ($\overline{W}$) = 15.9 g; $PC_k$ = 0.134.

**Modulus a was calculated using parameter A and protein concentration in dose ($a = A \cdot I \cdot PC_k$).

***Since ($t+2$) for complete antigen and fraction I was found to be different, accumulation of plasma cells was estimated as the arithmetic mean for two times.

****Deviation = $\frac{\text{experimental value − estimated value}}{\text{experimental value}} \times 100\%$.

## Conclusions

1. It was found that the concentration of antigen in blood and accumulation of cells of the plasmocyte class in CBA mice given intraperitoneal injections of capsular antigen (fraction I) of the pathogen of plague or complete antigen of the pathogen of tularemia were correlated, and the change from antigenic function to accumulation of plasma cells in a concrete experimental model could be described by a constant, parameter A.

2. A comparison of actual values of the plasmocyte reaction found in the experiment and estimates obtained by means of the average values of parameter A failed to demonstrate significant discrepancies.

3. The proposed equations and values for parameter A, which were obtained previously with administration of antigens separately, were tested in order to predict the level of the plasmocyte reaction in an experiment, where animals were given a mixture of different doses of soluble antigens; the estimates differed to some degree from experimental values, but these differences were relatively minor.

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# ISOLATION OF FRANCISELLA TULARENSIS FROM SIBERIAN LEMMINGS IN EASTERN TAYMYR

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[Article by L. S. Yegorova, V. A. Il'in, I. P. Algazin and G. B. Mal'kov, Omsk Scientific Research Institute of Endemic Infections]

[Text] Cases of tularemia among humans were observed in the Eurasian subarctic region in several parts of the Extreme North [6, 8, 9, 11, 15, 17, 18, 21, 22]. The pathogen of this infection was isolated from the Lepus timidus L. mountain hare [21, 22], Arvicola terrestris L. water vole, Microtus oeconomus Pall. root vole and Microtus middendorffi Poljak vole [11]. In addition, antibodies to F. tularensis were detected in Rangifer tarandus L. reindeer [3]. Epidemic outbreaks of tularemia occurred against the background of a large population of subarctic rodents. Cases of tularemia among humans in Scandinavia and the Kola Peninsula were preceded by mass scale deaths of Lemmus lemmus L. Norway lemmings [18, 20-22].

Many authors have described, long ago, epizootics among lemmings in years where there was a large number of rodents; however, in most cases, the etiology of these cases was undetermined. It has been established that lemmings are susceptible to tundra rabies and tularemia. The susceptibility of lemmings to tundra rabies, which is a widespread disease among wild and domestic animals of the Extreme North, was proven by serological examination of the brain of tundra rodents using the complement fixation test [CFT] [7]. It was possible to isolate the pathogen of this infection from the brain of lemmings who died after experimental infection, after 3-7 blind passages with change in species composition of laboratory animals. Spontaneous tularemia infection among lemmings was found in Scandinavia [20, 21]; heretofore, F. tularensis had not been isolated from lemmings in the territory of the Soviet North. Experimental studies demonstrated susceptibility of lemmings to F. tularensis on the example of L. lemmus, which [19] is classified in the first group of mammals that are highly susceptible and highly sensitive to tularemia [13].

The accumulated information about the probable involvement of lemmings in the spread of tularemia in the Extreme North warranted the assumption that there are endemic sites of tularemia of the tundra (lemming) type [10] and inclusion of the tundra region in the nosological range of tularemia [14].

We investigated the incidence of endemic infections in the Arctic in 1972-1973, in the summer (July, August) on the territory of the Taymyr National Okrug, Krasnoyarsk Kray, in the combined "North" expedition of the Siberian Branch of the USSR Academy of Medical Sciences. We selected regions of West and East Taymyr in the moss-lichen tundra subzone (72-73° north latitude) for our field studies and gathering material. In 1972, the work was conducted on the route along the Pyasina River (from the mouth of the Yangoda River to Pura River, 300 km route) and in 1973, at a permanent base in the Khatanga River basin (in the vicinity of the Novorybnoye trading post).

In this period, we trapped 498 lemmings and 2000 gamasid ticks. We used Gero traps, cylinders and our hands to catch the rodents. The standard method of trap-lines was used to record the number of lemmings, and the traps were equipped with a caprone [nylon-6] trip cord [2].

We took blood (unadulterated and on discs) and excised the spleen from the trapped lemmings, which were placed in a mixture of vaseline and paraffin [1]; the test tubes with spleens were stored in the refrigerator.

The lemming population of the Taymyr tundra is represented by two species, Siberian (Lemmus sibiricus Kerr.) and Arctic (Dicrostonyx torquatus Pall.), which constituted 96 and 4%, respectively, in our collections. It is known that very drastic changes in population size, with 2- or 3-year periods of fluctuations, are inherent in lemmings. Our observations of populations revealed that, after a profound "depression" of rodents in 1971 (according to the data of the Taymyr "Service of Yield of Game"), the number of lemmings started to increase in 1972 to an average of 7 specimens (in some areas up to 15) per 100 l.s. [not further identified]. In 1973, because of increased summer reproduction of rodents, there was a further rise in number of lemmings (up to 20, and in northern parts of the tundra up to 35 specimens).

In 1972, serological examination of 98 samples of lemming blood serum tularemia using the HANR [hemagglutination neutralization reaction] wi erythrocytic tularemia diagnosticum in a titer of 1:20 (++++) positive results were obtained in 4 cases (4.1%): in 2 Siberian (rodents caught near the mouth of Lyungfada River and Pura River, tributaries of Pyasina River) and 2 Arctic lemmings (caught along the Seliye River, tributary of Pura). Seropositive results were noted in animals that had spent the winter there and young specimens that appeared healthy, as well as in material from fresh rodent carcasses (2 positive cases for tularemia in Arctic lemmings, whose carcasses were discovered in an Arctic fox hole next to the Seliye River). Examination of spleens by the biological method (26 biological tests) in 1972 yielded negative results. The positive serological data led us to assume that there is circulation of F. tularensis among lemmings in Taymyr.

In 1973, we examined the spleens of 400 lemmings in 41 biotests on white mice (2 mice for each analysis). Cultures of F. tularensis were isolated from Siberian lemmings in 6 biotests. White mouse death occurred on the 5th-7th day. Necropsy of laboratory animals revealed the typical pathoanatomical changes for tularemia. Impression smears of organs from mice used in the biotests showed large quantities of F. tularensis. Organs (lymph nodes, spleen, liver) and blood were inoculated on coagulated yolk medium and, for a control, on beef-extract agar. After incubation for 24 h at 37° in the yolk medium, we observed growth inherent in Francisella tularensis; there was no growth of bacterial flora on agar. The culture showed the typical morphological signs of F. tularensis in smears stained according to Gram. All 6 strains were agglutinated by specific antitularemia serum (titer 1:3200) to one-half (++++) or a complete (++) titer.

Inoculation on medium with glycerin by the method of Yemel'yanova [5] revealed that the F. tularensis isolated from lemmings were referable to the Holarctic race; they did not ferment glycerin.

Table 1. Determination of virulence of F. tularensis isolated from organs of Siberian lemmings

| Laboratory animals | Dosage (bacterial cells) | Numb. of exper. animals | Died within 3-15 days | Survived |
|---|---|---|---|---|
| Rabbits | 1 billion | 3 | 3 | 0 |
| | 100 million | 3 | 0 | 3 |
| | 10 million | 3 | 0 | 3 |
| White rats | 1 billion | 5 | 5 | 0 |
| | 100 million | 5 | 1 | 4 |
| | 10 million | 5 | 0 | 5 |
| Guinea pigs | 10 | 3 | 3 | 0 |
| | 1 | 3 | 3 | 0 |
| | 0.1 | 3 | 1 | 2 |
| White mice | 10 | 5 | 5 | 0 |
| | 1 | 5 | 5 | 0 |
| | 0.1 | 5 | 2 | 3 |

The virulence of one of the cultures was tested on rabbits, white rats, guinea pigs and white mice (Table 1). F. tularensis isolated from Siberian lemmings did not differ from the F. tularensis strains isolated from other regions of the Soviet Union [4]. The other 5 strains were tested for virulence only on white rats and white mice, and they behaved similarly to the culture described above.

It must be noted that the tularemia culture was obtained from lemmings which appeared outwardly healthy. One strain of F. tularensis was isolated from material taken from a fresh Siberian lemming carcass.

The tularemia cultures were isolated from lemmings caught in the following habitats in Eastern Taymyr (Table 2): bottomland of Bludnaya River (5 km north of the Novorybnoye trading post) and lake-swamp troughs (4 km northeast of this inhabited center), which were low, very wet parts of the moss-lichen tundra.

In order to identify the possible vectors of F. tularensis, in 1973 we gathered and submitted to bacteriological examination gamasid ticks from lemming nests and from combing the animals.

Table 2. Location of lemmings and isolation of F. tularensis from them in different East Taymyr habitats

| Habitat | Number of lemmings caught | | L. sibiricus | D. torquatus | Number of isolated cultures |
|---|---|---|---|---|---|
| | absolute | % | | | |
| Regions near brooks | 117 | 29.3 | 117 | - | - |
| Bludnaya River floodplain | 86 | 21.5 | 86 | - | 2 |
| Lake-swamp regions | 192 | 48.0 | 192 | - | - |
| Ravines, "plakory" [turf-reinforced slopes?] | 5 | 1.2 | 2 | 3 | - |
| Totals | 400 | | 397 | 3 | 6 [sic] |

The species composition of gamasid ticks was relatively sparse. The most numerous representatives of the nest-burrow complex are widespread species--Hirstionyssus isabellinus Oudem. (obligate bloodsucking tick), Haemogasamus ambulans Thorell (facultative bloodsucking)--and the specific parasite of lemmings, the Laelaps lemmi Grube epizootic species.

We took for bacteriological tests gamasid ticks from the nests of lemmings--H. isabellinus (41%) and H. ambulans (33%). Before testing, the ticks were kept alive in the nests with maintenance of the proper microclimate. Rodent nests were collected in the same habitats where lemmings were trapped. The ticks were driven out by standard "thermoeclectors" [heat-jet emitters?].

A suspension was prepared from the gamasid ticks, which was given to two albino mice in a volume of 0.5 ml, subcutaneously. The mice died 6 days after infection. Necropsy revealed insignificant enlargement of regional lymph nodes, injected vessels in the subcutaneous cellular tissue, enlargement of the liver and spleen. We succeeded in detecting typical accumulations of F. tularensis in impression smears of the spleen stained according to Romanovsky-Giemsa, in addition to extraneous bacterial flora. When viscera were cultured on McCoy's yolk medium, no positive results were obtained due to depression of F. tularensis growth by the extraneous flora. We also failed to isolate a tularemia culture after the second and third passages on white mice.

Spontaneous tularemia infection of H. isabellinus, H. ambulans and L. lemmi has not been demonstrated in the Soviet Union; it was proven experimentally that H. isabellinus ticks are capable of transmitting and storing the pathogen of tularemia.

Thus, isolation of F. tularensis from lemmings in East Taymyr is indicative of the existence of tundra (lemming) tularemia sites in the Soviet Union. Gamasid ticks are probably involved in circulation and preservation of F. tularensis in tundra rodent populations. The absence of other rodents in typical tundra regions (the rodent population of the typical tundra is represented only by lemmings) and presence of an extensive zone without Ixodes ticks are indicative of substantial distinctions of endemic tularemia sites in the Extreme North.

## Conclusions

1. Tularemia in tundra rodents was demonstrated for the first time in the Soviet Union--bacteriologically in the Siberian lemming, serologically in the Siberian and Arctic lemmings.

2. The cultures of F. tularensis isolated from lemmings do not differ in properties from pathogens isolated in other regions of the Soviet Union.

3. Positive serological tests on Siberian and Arctic lemmings in a year of depressed rodent population and isolation of tularemia culture from Siberian lemmings in a year of growth in number of lemmings are indicative of continuous circulation of the pathogen of this infection in populations of tundra rodents.

4. Bacterioscopic detection of F. tularensis in gamasid ticks is indicative of the probable involvement of representatives of the nest-burrow complex in transmission of the pathogen of tularemia among lemmings in tundra sites of this infection.

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# EXPERIMENTAL STUDY OF MIXED TULAREMIA INFECTION AND OMSK HEMORRHAGIC FEVER IN MUSKRATS

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[Article by N. B. Dunayev, L. S. Yegorova and T. N. Fedorova, Omsk Scientific Research Institute of Endemic Infections]

[Text] In 1963, mixed epizootics of Omsk hemorrhagic fever (OHF) and tularemia were discovered on some lakes of West Siberia (among muskrats) [3, 4 and others]. The data in the literature [1, 2, 6 and others] warrant the conclusion that some infections can, in certain combinations, cause deviations in the course of tularemia in wild and laboratory animals. From the standpoint of epizootiology and epidemiology, the possibility of aggravation of the infectious process, which would be instrumental in involving animals in the epizootiological process, as well as the possibility of manifestation of heterologous immunity to tularemia [2, 5], are of considerable interest.

We tried to track the course of each of these infections in animals under experimental conditions after combined infection with the pathogens of tularemia and OHF.

Before starting the experiments, the experimental animals (muskrats) were in the laboratory for 2 months. We did not observe any nonspecific deaths during this period. We took 18 muskrats for each experiment. The same number of control animals was infected separately by the same doses of pathogens of OHF and tularemia.

For infection, we used the Balangul' strain of OHF, which had been submitted to numerous passages (log $LD_{50}/m\ell$ was 8.5 with intracerebral infection) and virulent F. tularensis strain No 503. OHF virus was injected subcutaneously in the inguinal region of the left hind leg in a dosage of 100 $LD_{50}/m\ell$, and F. tularensis subcutaneously in the right hind leg in a dosage of 1 bacterial cell (according to the optical standard of the State Institute for Standardization and Control of Medical Biologicals). We sacrificed 2 animals 6 h after infection, then the same number every other day to the end of the experiment, after having collected blood from the heart for virological, microbiological and serological tests. The animals were dissected, and cultures on yolk medium were made of lymph nodes and parenchymatous organs. In addition, we prepared impression smears for bacterioscopy and testing by the method of fluorescent antibodies (MFA). Organs were used for biological tests on white mice (weighing 6-8 g), which were infected intracerebrally for demonstration of virus. We determined presence of antibodies to OHF virus in muskrat blood using the hemagglutination depression reaction and to F. tularensis with the agglutination reaction. The results of blood tests on the muskrats before the experiment were indicative of absence of antibodies to these pathogens.

In the first experiment, the animals were infected with the above-mentioned pathogens. Clinical signs of disease appeared on the 6th-7th day. The animals became sluggish, inactive and presented suppuration of the eyes; some muskrats developed paresis of the extremities, and one died 8 days after infection.

The virus was demonstrated in whole blood 1 day after infection. Viremia reached a maximum ($10^{-6}$) after 3 days. At the end of the experiment virus titer in blood constituted $10^{-3}$. Virus-specific antibodies were found in a titer of 1:20 2 days after infection. At the end of the experiment antibodies were demonstrable in a titer of 1:40 (Table 1).

Table 1.
Results of virological tests on muskrats when infected with pathogens of OHF and tularemia

| Infection | Day of post-infection examination | Blood dilution at which virus was isolated | Titer of antibodies to OHF virus found in animals | |
|---|---|---|---|---|
| | | | № 1 | № 2 |
| Simultaneous | 6 h | 0 | 0 | 0 |
| | 1 | 10 | 0 | 0 |
| | 2 | $10^{-1}$ | 1:20 | 1:20 |
| | 3 | $10^{-4}$ | 1:40 | 1:40 |
| | 4 | $10^{-6}$ | 1:80 | 1:80 |
| | 5 | $10^{-5}$ | 1:80 | 1:40 |
| | 6 | $10^{-5}$ | 1:20 | 1:40 |
| | 7 | $10^{-5}$ | 1:80 | 1:160 |
| | 8 | $10^{-3}$ | — | 1:40 |
| Successive | 3 | $10^{-6}$ | — | 1:10 |
| | 4 | $10^{-8}$ | 1:20 | 1:20 |
| | 5 | $10^{-6}$ | 1:20 | 1:10 |
| | 6 | $10^{-5}$ | 1:40 | — |
| | 7 | $10^{-4}$ | 1:10 | 1:20 |
| | 8 | $10^{-1}$ | 1:160 | 1:160 |
| | 9 | $10^{-2}$ | 1:80 | — |

Note: 0--no virus isolated from whole blood; no antibodies found in 1:10 serum dilution.

Direct inoculation of blood and organs on coagulated yolk medium enabled us to isolate cultures of F. tularensis 3 days after infection from the right inguinal and submaxillary lymph nodes and spleen, but only after 6 days from blood. Thereafter, the pathogen was isolated regularly from these organs to the end of the experiment. In the biotest on white mice, the pathogen of tularemia was demonstrable in the inguinal lymph node 6 h after infection, in the submaxillary lymph node after 1 day and in the spleen and blood after 2 days. Thereafter, we were able to demonstrate it in all examined organs. Examination of the preparations by the MFA confirmed the results of the biotests (Table 2). Serologically, antibodies to F. tularensis in a titer of 1:10 (++) were demonstrated 8 days after infection.

Dissection of animals 6 h after infection failed to reveal visible changes in organs; at the site of injection of infectious material, there was dilatation of blood vessels on both the left and right sides after 1 and 2 days; enlargement of the spleen was found after 3 days. Thereafter, there was progression of visceral changes. After 5 days, necrotic foci appeared in the spleen, the lymph nodes were considerably hyperemic and enlarged. Subsequently, necrotic foci also appeared in the liver, and the intestine showed petechial hemorrhages.

In addition to the above-mentioned changes in internal organs, 8 days after infection we demonstrated necrosis of the inguinal lymph node.

Table 2. Results of tests for tularemia after infection with pathogens of OHF and tularemia

| Infection | Postinfect. day of testing | Presence (+) of F. tularensis when tested | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | bacteriolog. | | | | biologically | | | | MFA | | | |
| | | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| Simultaneous | 6 h | | | | | + | | | | + | | | |
| | 1 | | | | | + | + | | | + | + | | |
| | 2 | | | | | + | + | + | + | + | + | + | |
| | 3 | + | + | + | | + | + | + | + | + | + | + | |
| | 4 | + | + | + | | + | + | + | + | + | + | + | |
| | 5 | + | + | + | | + | + | + | + | + | + | + | |
| | 6 | + | + | + | + | + | + | + | + | + | + | + | + |
| | 7 | + | + | + | + | + | + | + | + | + | + | + | + |
| | 8 | + | + | + | + | + | + | + | + | + | + | + | + |
| Successive | 6 h | | | | | | | | | | | | |
| | 1 | | | | | + | + | | | + | | | |
| | 2 | | | | | + | + | + | | + | + | + | |
| | 3 | + | | | | + | + | + | | + | + | + | |
| | 4 | + | + | + | + | + | + | + | + | + | + | + | + |
| | 5 | + | + | + | + | + | + | + | + | + | + | + | + |
| | 6 | + | + | + | + | + | + | + | + | + | + | + | + |

Key: 1) inguinal lymph node 3) spleen
2) submaxillary lymph node 4) blood

A comparison of the results of virological, microbiological and serological tests on animals with single and mixed infection shows that viremia differed only in dynamics of development, but at the end of the experiment it was the same in both groups of animals ($10^{-3}$). Delayed production of antibodies to OHF virus was a distinction of mixed infection: after 8 days, the antibody titer was 1:40, whereas in animals infected only with OHF it was 1:160. There was more intensive development of bacteremia in the case of mixed infection: F. tularensis was demonstrated in the spleen 1 day sooner and in blood 2 days sooner than with monoinfection.

In comparing these data to the morphological changes in animal organs, we must mention the more serious course of mixed infection. Muskrats died of tularemia 2 days earlier and of OHF 3-5 days earlier. In our opinion, this was caused by the more serious course of OHF, rather than tularemia, although the opposite could have been assumed according to microbiological data (early appearance of bacteremia). Morphologically, this was indicated, first of all, by 2-day delay in appearance of specific signs of tularemia in animals infected with both pathogens. At the same time, there were more serious vascular lesions: earlier appearance of inflammation and involvement in the process not only of small, but large vessels. The slower production of antibodies to OHF virus in the case of mixed infection also confirmed the assumption of more severe course of OHF.

Thus, concurrent infection with pathogens of OHF and tularemia elicited parallel development of both infections in muskrats, a more severe course of infection related to more intensive vascular involvement under the effect of OHF virus and inhibition of tularemia due to development of OHF.

In the second experiment, the muskrats were infected with OHF virus and, 3 days later, F. tularensis. Clinical symptoms of disease appeared in the animals 3 days after infection with F. tularensis, i.e., 6 days after infection with OHF virus, and they were similar to symptoms observed in the first experiment. One muskrat died 5 days after F. tularensis infection and 4 more 6 days after infection. The rest of the animals presented paresis and paralysis of the limbs on the same days.

Virological blood tests revealed that viral titer reached a maximum ($10^{-6}$) 3 days after OHF infection and held at this level for another 2 days, after which it dropped to $10^{-4}$ and remained on this level to the 8th day, dropping to $10^{-3}$ by the end of the experiment (see Table 1).

Bacteriological examination enabled us to isolate a culture of F. tularensis 3 days after infection from the inguinal lymph node. Thereafter, the pathogen of tularemia was regularly isolated from all organs examined (see Table 2).

With the biotest on white mice, we were able to detect F. tularensis in the inguinal and submaxillary lymph nodes 1 day after infection, in the spleen 2 days after and also in blood 3 days after. Thereafter, F. tularensis was consistently isolated from all mentioned organs and blood to the end of the experiment. The results of the test by the MFA essentially confirmed the results of the biological method (see Table 2).

The macroscopic changes in internal organs upon dissection 6 h after infecting the muskrats with F. tularensis were found to be the typical ones for the initial stage of development of OHF: hyperemia of the left inguinal lymph node, dilatation of intestinal blood vessels, petechial hemorrhages in the intestine and other organs. After 1 day, similar changes were demonstrated in the organs, whereas after 2 days we also found changes in lymph nodes on the right side, in the form of enlargement and hyperemia. In addition, the spleen was enlarged, fine necrotic sites appeared in the intestine, hepatization in the lungs and vasodilatation in the brain (brain tissue was plethoric on section). Thereafter, the visceral changes progressed, with prevalence of changes inherent in OHF in some muskrats, changes inherent in both OHF and tularemia in others.

Thus, our investigation revealed that infection of muskrats first with OHF virus and 3 days later with F. tularensis virtually failed to elicit deviations in development of viremia, as compared to the control; as with control infection of muskrats with OHF virus, we observed elevation of blood virus titer to maximum values 3-4 days after infection, after which it gradually declined. The difference between experimental and control titers was insignificant 9 days after infection.

Our study of development of the tularemia process enabled us to demonstrate some delay in its development on the 1st postinfection day, which was confirmed bacteriologically, biologically and MFA results. This is attributable to the fact that, in this case, as in the first experiment, there was depression of the infectious process elicited by F. tularensis due to development of OHF. Macroscopically the visceral changes were typical of both OHF and tularemia.

Consequently, infection with the pathogen of tularemia 3 days after administration of OHF virus aggravated the course of the infectious process and led to death of animals due to mixed infection; the muskrats died 2 days earlier than in the control when infected with OHF virus alone and 4-5 days sooner than in the control when infected only with F. tularensis.

Conclusions

1. Muskrats developed mixed infection when infected with the pathogens of tularemia and Omsk hemorrhagic fever.

2. The more severe course of mixed infection was attributable to development of vascular lesions under the influence of OHF virus.

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# DIAGNOSTIC PROPERTIES OF BRUCELLOSIS AND TULAREMIA ANTIGENIC ERYTHROCYTE DIAGNOSTICUMS

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[Article by B. P. Tsybin, I. F. Taran and A. I. Tinker, Scientific Research Plague-Control Institute of the Caucasus and Transcaucasia, Stavropol]

[Text] Among the serological methods used to diagnose infectious diseases and examine the qualitative aspects of the immunological response, the passive hemagglutination reaction (PHAR) and various modifications thereof have gained deserved recognition. However, this test can be used in broad practice only for infectious diseases, for the diagnosis of which commercial production of standard erythrocyte diagnosticums has been set up. As for brucellosis and tularemia, methods of recovering the appropriate preparations have not yet gone beyond the stage of laboratory testing.

Vershilova et al. [1] demonstrated that antigens recovered from brucella by different methods are not the same in either serological activity or capacity to sensitize erythrocytes. The polysaccharide extracted from brucella by the Boivin method and then submitted to alkaline hydrolysis is the most suitable for sensitization of erythrocytes. Antigenic erythrocytic diagnosticum for tularemia was also obtained by the Boivin method [4]. A test of the diagnostic features of these diagnosticums was indicative of their high sensitivity, specificity and stability. However, the relatively complicated and time-consuming Boivin method of recovering specific antigens, as well as the insignificant yield obtained, render it unacceptable to use these methods for production of brucellosis and tularemia antigenic erythrocyte diagnosticums on a commercial scale.

For this reason, we have developed methods of obtaining antigenic erythrocyte diagnosticums for brucellosis and tularemia, which can be recommended for commercial production. We are submitting here the results of studying the diagnostic qualities of these products.

For the PHAR for brucellosis, we used erythrocytes loaded with a lipopolysaccharide-protein complex [5] from Br. abortus vaccine strain 19 by means of secondary sodium alkylsulfate ($R = OSO_3 = Na$). The titer in the PHAR of different series of formalin-treated erythrocytes, which were sensitized with the above antigen, with homologous hyperimmune agglutinating serum ranged from 1:51,200 to 1:102,400 (in the agglutination test the titer was 1:3200). The diagnosticum retained its activity for 8 months and, in dry form, up to 1 year (observation period). In all, we prepared 37 series of antigenic erythrocytic diagnosticum for brucellosis, and the total amount thereof was about 46 ℓ. The quality of the produced series of diagnosticum was stable.

We tested the specificity of diagnosticum in the PHAR with hyperimmune heterologous sera and with serum from people and animals known to be in good health, as well as from people with various infectious diseases. A positive reaction in low titers was obtained with most standard hyperimmune heterologous sera (1:10-1:80), and it was positive up to a titer of 1:320 only with cholera and tularemia sera. In the latter case, the positive result could be attributed to the fact that the structure of surface antigenic determinants is the same in the pathogens of brucellosis, tularemia and cholera. In the PHAR with serum from people and animals immunized against tularemia and those who had had this infection, using brucellosis antigenic erythrocyte diagnosticum , the titer did not exceed a dilution of 1:80. In the PHAR with 646 sera from people known to be in good health, as well as animals, the results were negative in all cases. Testing of 122 blood serum samples from people with various infectious diseases revealed positive reactions in 52 cases, with titers of 1:10 to 1:80. All these data served as grounds to consider serum dilution of at least 1:100 to be a diagnostic titer for the PHAR.

We conduct the studies and evaluated the diagnostic properties of different series of brucellosis erythrocyte diagnosticum using the PHAR on blood serum of people and animals at different stages of the vaccinal and infectious brucellosis process. In order to compare the diagnostic value of the PHAR to conventional serological tests, as well as check the reliability of PHAR results, we ran concurrently the agglutination reaction (RA), complement fixation reaction (CFR) and reaction of prolonged complement fixation (RPCF) using conventional methods and standard commercial antigens. In all, we tested 8515 serum samples, 1136 of which were from humans, 1196 from guinea pigs and 6180 from cattle.

In all groups, the incidence of positive PHAR results was higher than with use of AR, CFR and RPCF, which was indicative of the greater sensitivity of PHAR (Table 1). At the same time, on the basis of the percentage of positive PHAR, CFR and RPCF, it can be stated that these reactions were virtually equivalent in diagnostic qualities; however, the appreciable difference in labor required to run these tests was in favor of the PHAR.

In the course of our study, we found that normal hemagglutins (in titers of 1:10 to 1:80) to unsensitized ram erythrocytes were encountered in blood serum from healthy, sick and immunized farm animals. Preliminary absorption of such sera by erythrocytes eliminated completely the positive reactions starting with a dilution of 1:40. In order to reduce time and labor involved in the PHAR, one can test serum without preliminary adsorption, but with mandatory checking of the reaction with unsensitized erythrocytes and the tested serum. If control erythrocytes are agglutinated by the tested serum, it must be adsorbed and retested. In this regard, it should be noted that, even with a negative check with unsensitized erythrocytes and unadsorbed tested serum, the result in the PHAR could be 1-2 dilutions higher due to the fact that, in this serum, normal hemagglutinins contained in subliminal doses emerge as an additional load to specific hemagglutinins and increase the reaction titer.

Table 1. Results of serological testing of different groups of animals for brucellosis

|  | Animal group | Number of animals tested | PHAR |  | AR |  | CFR | RPCF |
|---|---|---|---|---|---|---|---|---|
|  |  |  | positive results, % | geometric mean of titers | positive results, % | geometric mean of titers | positive results, % |  |
| At farms with no brucellosis problem | Cattle [large] |  |  |  |  |  |  |  |
|  | 50 days after immunization | 480 | 98,3 | 898 | 96,0 | 179 | 97,8 |  |
|  | 1 year after immunization | 846 | 84,0 | 184 | 47,0 | 63 | 80,0 |  |
|  | 2 years after reimmunization | 574 | 50,3 | 106 | 25,0 | 57 | 33,7 |  |
| Farms with brucellosis problem | Cattle [large] | 1527 | 58,9 | 326 | 34,3 | 98 | 53,8 |  |
|  | Goats and sheep | 1960 | 69,4 | 364 | 57,2 | 116 | 62,3 | 62,9 |

In our tests, the mean PHAR titer without preliminary adsorption constituted 1:686 for 248 serum samples with negative checks with unsensitized erythrocytes. After adsorption of sera with 50% suspension of formalin-treated erythrocytes, the mean titer in the PHAR dropped to 1:296. These data indicate that, when running the PHAR, adsorption of serum by erythrocytes should be one of the mandatory factors that rules out nonspecific readings and yields more reliable data.

We then determined the diagnostic value of the PHAR with the above-mentioned diagnosticum in studying the immunological structure of people living in areas with a brucellosis problem. We screened 1026 people in all: 160 with chronic brucellosis, 574 who were immunized against brucellosis and 292 who were not immunized (Table 2). In 89% of those who were immunized against brucellosis, the Wright reaction was negative, whereas when these sera were tested in the PHAR, positive reactions were obtained with titers that were reliable in specificity (over 1:100) in 32% of the cases. We also demonstrated positive reactions among people who did nor receive brucellosis inoculations. This indicated that the working and living conditions of these people had led to brucellosis infection. These data warrant recommending the PHAR for studies of immunological structure of the public in order to detect infection in groups of people who were exposed to different conditions of contact with sources of Brucella.

Testing by the PHAR of 160 samples of blood serum from people with chronic brucellosis yielded positive results in 64%, whereas the Wright reaction was positive in only 8%.

We also tested the antibody neutralization reaction (ANR). We used the method of Levi and Momot [2] to run this test. Specificity was checked with 5 species of heterologous microorganisms (pathogens of tularemia, cholera, plague, typhoid fever, dysentery). It was established that the brucellosis erythrocyte diagnosticum detected 2 million to 1 million homologous bacterial cells in the ANR. Positive results were not obtained in any cases in the tests with heterologous species of microorganisms, which was indicative of specificity of this diagnosticum.

Table 2. Results of serological testing of people living in areas with brucellosis problem

| Groups screened | Quantity of samples tested | PHAR | | AR | |
|---|---|---|---|---|---|
| | | posit. % | titer geom. mean | posit. % | titer geom. mean |
| Livestock farmers: | | | | | |
| total | 524 | 36,2 | 352 | 11,8 | 100 |
| immunized | 472 | 37,7 | 486 | 11,8 | 124 |
| not immunized | 52 | 23,0 | 217 | 11,5 | 78 |
| People in other occupations: total | 342 | 4,3 | 156 | 3,2 | 45 |
| immunized | 102 | 8,8 | 166 | 4,9 | 42 |
| not immunized | 240 | 2,5 | 147 | 2,5 | 47 |
| Patients with chronic brucellosis | 160 | 64,3 | 738 | 8,1 | 235 |

When we used the ANR to test organs of 246 guinea pigs, which were infected with different doses of Brucella, the results were positive in 23% of the cases, with negative results of bacteriological test, and in 48% of the cases a positive ANR coincided with culturing of Brucella from organs. The range of delay in the reaction was 1:10 to 1:160.

Thus, our testing of the brucellosis antigenic erythrocyte diagnosticum, which we prepared, in a mass screening of people and animals confirmed its stability, high sensitivity and specificity. A check of the diagnostic value of the diagnosticum using the ANR was also indicative of its high sensitivity and specificity. All this can serve as grounds for more extensive use of this product in practical brucellosis diagnostication.

To prepare the antigenic erythrocyte diagnosticum for tularemia, we used a specific antigen recovered by treating commercial tularemia diagnosticums with a surfactant--secondary sodium alkylsulfate. For this purpose, we added 2% of secondary sodium alkylsulfate to the tularemia diagnosticum in its original state and left it at room temperature for 1 h, mixing it periodically. The suspension was then centrifuged for 1.5-2 h at 6000 r/min; the supernatant was decanted and combined with an aliquot of 5% suspension of erythrocytes treated with formalin according to Vaynbakh in the modification of Men'shov and Shmuter [3]. After exposure to 37° for 16-18 h, the erythrocytes were eluated 3 times in saline, with centrifugation at 1500 r/min for 10 min. The sediment of erythrocytes was resuspended in saline to a 2.5% concentration and used in the PHAR. We added 5% formalin to the diagnosticum as preservative.

The hemagglutination reaction with erythrocytes sensitized by tularemia antigen as described above and standard agglutinating tularemia serum was positive in a titer of 1:204,800 (in the AR the titer was 1:1600). The original activity of the 26 series of antigenic erythrocyte diagnosticums for tularemia that we prepared did not diminish for 7-8 months and, in a dry state, for up to 1 year (duration of observation period).

We determined the diagnostic properties of the tularemia antigenic erythrocyte diagnosticum in a comparative test of serum obtained from 486 animals with experimental tularemia, using the AR and PHAR. In all cases, the serum titer was 7-8 dilutions higher in the PHAR than AR. The range of specificity of the diagnosticum was tested with the PHAR using blood serum from people known to be in good health, as well as with various infectious diseases (brucellosis, typhoid fever, dysentery, etc.). In all cases, the results of testing 236 serum samples by the PHAR with tularemia diagnosticum were negative.

We also checked the specificity of the diagnosticum in the ANR with homologous and different species of heterologous microorganisms (pathogens of brucellosis, cholera, plague, typhoid fever, dysentery). It was established that the tularemia erythrocyte diagnosticum detected 4 million to 1 million homologous bacterial cells in the ANR. At the same time, the results of the ANR with different species of heterologous microorganisms were negative in all cases, which was indicative of specificity of this diagnosticum.

Use of the ANR to test organs of 164 animals experimentally infected with tularemia yielded a positive result in 86 cases: a delayed reaction was observed in dilutions of 1:10 to 1:256, with negative results in the bacteriological test. Use of the ANR to test 524 pellets from birds collected in an endemic site for tularemia yielded positive reactions in 34 cases, in dilutions of 1:10 to 1:164.

Thus, the antigenic erythrocyte diagnosticum for tularemia is highly sensitive, specific and stable. A comparative study of diagnostic properties of diagnosticum prepared by our method and diagnosticum produced by Meshcheryakova revealed that these products are equal in sensitivity and specificity with the PHAR and ANR; however, as we have indicated above, extraction of antigen by the Boivin method is relatively difficult and time-consuming, whereas our method is simpler and yields much more specific antigen from bacterial mass, which ultimately lowers the cost of the product.

## Conclusions

1. The antigenic erythrocytic preparations for diagnosing brucellosis and tularemia, which were produced by our method, were found to be highly sensitive, specific and stable in a mass-scale serological study of people and animals at different stages of the vaccinal and infectious process.

2. The simplicity of recovering specific antigens to be used for sensitization of formalin-treated erythrocytes and consistency of the results of using the methods of preparing antigenic erythrocyte diagnosticums enables us to recommend the described methods for use on an industrial [commercial] scale.

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# DETECTION OF FRANCISELLA TULARENSIS IN ANIMAL ORGANS AT EARLY STAGES OF DEVELOPMENT OF INFECTION

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[Article by N. G. Olsuf'yev, Ye. V. Ananova, I. S. Meshcheryakova and R. A. Savel'yeva, Institute of Epidemiology and Microbiology imeni Gamaleya, USSR Academy of Medical Sciences, Moscow]

[Text] The existing test methods make it possible to detect the pathogen of tularemia in infected animals with adequate accuracy. The biological test on white mice is the most sensitive and reliable method of detecting F. tularensis. However, even with a large infective dose, the mice die only on the 3d-4th day, whereas with use of a small number of bacteria for infection they die on the 7th-9th day or later.

In order to speed up the answer, one can sacrifice tested animals at the early stage of development of the infectious process, rather than wait for them to expire, and examine their organs for the pathogen. In this case, the results can be obtained several days sooner. The method of fluorescent antibodies (FA), which was developed to apply to testing for F. tularensis by several authors [1, 2, 4, 5, 10, 11, 13], must be deemed particularly promising for this purpose. The advantage of this method is that it is quite sensitive and results are obtained quickly (within 1.5-2 h). It was previously shown [1] that with the FA method the bacterium can be detected in white mouse organs 4 days after being infected subcutaneously with 10 bacterial cells. The culture method can also be used to isolate the pathogen within the same time.

However, there are indications in the literature of earlier detection of F. tularensis in white mouse organs--3 days [11] and even 18 h [3] after infection with a minimal dose.

In recent years, serological reactions based on the use of erythrocyte diagnosticums have been used with success for demonstration of F. tularensis in animal organs. The antibody neutralization reaction (ANR) in the presence of antigenic erythrocyte diagnosticum was developed [6, 8], and it was shown that the minimum demonstrable dose of antigen constitutes 1 million bacterial cells. Use of an antibody erythrocytic diagnosticum [9] in the passive hemagglutination reaction (PHAR) permits demonstrations of thousands of bacterial cells.

Our objective here was to detect F. tularensis at the early stages of the infectious process (with minimal infection doses) using a set of bacteriological and serological methods.

We used 156 white mice weighing 16-18 g in the experiments. The animals were infected subcutaneously with strains No 503/834 (Holarctic race) and Schu (Nearctic race), which were given in 3 doses: 1, 10 and 100 bacterial cells according to the standard of the Institute imeni Tarasevich. The cultures to be used for infection were grown for 48 h on coagulated yolk medium. The animals were sacrificed 1, 2, 3 and 4 days after infection. At each tested time and for each infective dosage we used 5 (for the Schu strain) or 8 (for strain No 503/834) animals.

Upon dissection of sacrificed animals, we recorded the condition of internal organs and examined tissue at the site of infection, including regional lymph nodes and the spleen. We used the following methods: inoculation on nutrient media, immunofluorescence test, PHAR with antibody and ANR with antigenic erythrocyte diagnosticums. Cultures were made on coagulated yolk medium and Yemel'yanova's medium (fish-yeast agar with cystine, glucose and 10% defibrinated horse blood).

The cultures were kept at 37° for 10 days, observing growth and time of its appearance.

For the immunofluorescence study, we prepared impressions of a tissue section from the site of infection, together with regional lymph nodes and spleen, on a thoroughly defatted slide. In addition to the impressions, we prepared smears from homogenates of the same tissues. The preparations were air-dried at room temperature, then fixed in 96° alcohol for 20 min. Preparations from mice infected with different doses and at different times were fixed separately to preclude the possibility of accidental transfer of some bacteria from one slide to another. The fixed preparations were treated with a mixture of fluorescent tularemia serum and bovine albumin labeled with rhodamine sulfofluoride, used in one dilution less than the working dilutions (both are produced by the Institute of Epidemiology and Microbiology imeni Gamaleya). This mixture was applied to a slide with an impression and it was "stained" for 20 min in a humid chamber at room temperature. Subsequent treatment of the preparation and examination were performed by conventional methods. The presence of a few fluorescent bacteria in several fields of vision was considered as the minimal criterion of a positive result.

For testing in the PHAR and ANR, tissue from the infection site and the spleen were ground in a porcelain mortar, with 2 mℓ saline added; we arbitrarily considered the initial suspension to be a 1:40 dilution for tissue from the infection site and 1:20 for the spleen. For the purpose of decontamination and fuller extraction of antigen, the obtained suspensions were boiled in a water bath for 10-15 min.

The supernatant was filtered through an asbestos filter, and for removal of heterologous antibodies to ram erythrocytes the filtrates were adsorbed, adding 50% formalin-treated erythrocyte suspension at the rate of 0.1 mℓ/mℓ test-tube contents. After leaving it to stand (for at least 1 day), the supernatant was tested using the ANR with antigenic diagnosticum and PHAR with antibody diagnosticum using conventional methods.

we failed to demonstrate differences in time of detection fo F. tularensis in mice between the experiments with No 503/834 and the Schu strain (see Table); for this reason, the results submitted below were summated.

Results of testing white mice for Francisella tularensis

| Time of testing<br>Time of testing | Infective dose (quantity of bacterial cells) | Strain No 503/834 | | | | | | | | Schu strain | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | tissue from site of infection | | | | spleen | | | | tissue from site of infection | | | | spleen | | | |
| | | culture | FA | PHAR | ANR | culture | FA | PHAR | ANR | culture | FA | PHAR | ANR | culture | FA | PHAR | ANR |
| 1st day | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| | 10 | – | ± | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| | 100 | ± | ± | – | – | – | – | – | – | ± | – | – | – | – | – | – | – |
| 2d day | 1 | ± | – | – | – | ± | – | – | – | ± | – | – | – | – | – | – | – |
| | 10 | ± | ± | – | – | ± | ± | – | – | + | ± | – | – | ± | ± | – | – |
| | 100 | ± | ± | ± | – | ± | ± | – | – | + | + | ± | – | ± | – | – | – |
| 3d day | 1 | ± | ± | ± | – | ± | – | ± | – | + | ± | – | – | + | ± | ± | – |
| | 10 | ± | + | + | ± | + | ± | ± | – | + | + | – | – | + | + | – | – |
| | 100 | + | + | + | + | + | + | ± | – | + | + | ± | ± | + | + | ± | ± |
| 4th day | 1 | + | + | + | + | + | + | ± | – | + | + | ± | – | + | + | ± | – |
| | 10 | + | + | + | + | + | + | + | ± | + | + | ± | ± | + | + | ± | ± |
| | 100 | + | + | + | + | + | + | + | ± | + | + | ± | ± | + | + | ± | ± |

Key: +) positive results in all cases
±) positive results in some of the cases
-) negative results in all cases

The pathogen was not demonstrable in any of the cases 1 day after infection with 1 bacterial cell. After infection with 10 bacteria, the pathogen was found in only 2 out of 13 cases and after infection with 100 bacteria, in 6 out of 13, mainly in culture and partially by the FA method. There was considerable rise in incidence of demonstration of the pathogen after 2 days, but it constituted 100% only after infection with 100 bacterial cells, whereas with use of 1 bacterial cell there were 5 findings out of 13 cases and with use of 10 bacterial cells 12 findings out of 13. At this time, the pathogen was also demonstrated mainly in culture, much less by the FA method and in only 2 cases (out of 13) was the PHAR positive after infection with 100 bacteria. After 3 days, the pathogen was demonstrated in all tested mice infected with 10 and 100 bacterial cells (13 for each dose) and in 11 out of 13 mice infected with 1 bacterial cell. There was an increase not only in number of positive findings, but in amount of pathogen in the examined tissue.

After 4 days, the pathogen was found in all mice, regardless of infective dosage (total of 38 mice tested) and by virtually all of the methods used. Very high titers were demonstrable with the PHAR and ANR, up to 1:320,000 and 1:5120, respectively. At this time, many fluorescent bacteria were demonstrated by the FA method.

In most cases, F. tularensis was found both at the site of infection and in the spleen, particularly at the late testing times. But, in some cases, the pathogen was isolated only from tissue at the site of infection and seldom only from the spleen.

The largest number of positive results were referable to cultures, and growth was demonstrated slightly more often on Yemel'yanova's medium than coagulated yolk medium. In all, we obtained 354 cultures, 195 in the former medium and 159 in the latter. Growth was usually observed after 2-3 days, but in a number of cases later and, occasionally, as early as 1 day after inoculation. The FA method is in second place according to incidence of demonstration of the pathogen; with it, F. tularensis was found in 101 cases. Positive results with the PHAR and ANR were observed from the 3d day on (very seldom after 2 days), and the most consistently when large doses--10 and 100 bacterial cells--were used for infection.

It should be mentioned that when the mice were infected with the strain of the Nearctic race we observed somewhat less accumulation of the pathogen in them, as compared to infection with strain No 503/834 of the Holarctic race. This was manifested by a smaller quantity of bacteria according to the FA method, as well as results of PHAR and ANR.

Examination of material from mice infected with 10 and 100 bacteria of the Schu strain showed positive reactions only in some cases after 4 days, and the titers were considerably lower than in mice infected with No 503/834. Thus, the PHAR with tissue from the infection site was positive in less than half the mice in titers of 1:80-1:640, and with spleen tissue in titers of 1:40-1:2560. The ANR with tissue from the site of infection was positive in titers of 1:60-1:320. These findings could be related to the distinction of pathogenesis of infection when using F. tularensis of different geographic races [7].

As we know, corticosteroids lower natural resistance, creating the conditions for rapid reproduction and massive contamination of organs and tissues with a pathogen. In view of this, we conducted an additional experiment on animals infected with the same strains, but after preliminary hydrocortisone treatment. We used the Hungarian product of the Richter Firm in a dosage of 5 mg per white mouse, giving it once hypodermically 5 h before infecting them with F. tularensis.

In this experiment, we used two infective doses, 1 and 10 bacterial cells, since with 100 bacteria there was intensive development of the pathological process with massive contamination of organs and tissues with the pathogen even in mice untreated with hydrocortisone.

Otherwise, we used all of the above-described methods in this experiment.

As shown by the findings, there was no appreciable difference between mice treated with hydrocortisone and intact animals with regard to time of demonstration of the pathogen. Our results coincide with data in the literature [11]. Some differences were noted only in the group of animals infected with the Schu strain, as compared to the analogous group that was not given hydrocortisone. Thus, the FA method demonstrated more accumulation of the pathogen than in animals not treated with hydrocortisone. This was also reflected in the PHAR and ANR results. These reactions were positive in all experimental mice on the 3d and 4th days, and in high titers: with tissue from infection site ANR in titers of 1:160-1:1280 and PHAR 1:1280-1:20,000, with spleen tissue 1:160-1:2560 and 1:320-1:80,000, respectively.

Analysis of our data shows that, with hypodermic infection of white mice with minimal doses of 1 and 10 bacterial cells (according to State Control Institute of Medical Biologicals imeni Tarasevich) of Holarctic or Nearctic race, the pathogen was consistently demonstrable in animals with all methods used 3 days after infection, positive findings were irregular after 2 days and isolated after 1 day. With use of the method of FA of impression smears, a larger number of positive results was obtained than with testing of smears of organ homogenates.

Thus, our data concerning the time of detection of the bacterium in white mouse organs in the case of low infective doses are consistent with the results of Franek [12] and do not confirm the data of Gulida [3], who recommended that mice be tested in analogous cases 1 day after infection.

## Conclusions

1. Bacteriological and serological tests make it possible to consistently demonstrate F. tularensis in white mouse organs 3 days after hypodermic infection with minimum doses; earlier detection of the pathogen is possible in isolated cases and only by methods of culture and FA.

2. Use of serological tests with erythrocytic diagnosticums provides a quantitative characteristic: accumulation of bacteria in animal organs.

3. We failed to demonstrate appreciable differences between infection with Holarctic and Nearctic strains of F. tularensis, as well as pretreatment of mice with hydrocortisone, with regard to time of detection of the pathogen.

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# USE OF IMMUNOFLUORESCENCE METHOD FOR DETECTION OF F. TULARENSIS IN DEVELOPING CHICK EMBRYOS

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[Article by R. I. Kudelina and Ye. V. Ananova, Institute of Epidemiology and Microbiology imeni Gamaleya, USSR Academy of Medical Sciences, Moscow]

[Text] In recent years, the immunofluorescence method has been considered rather promising as a method of high-speed laboratory diagnosis, which permits rather reliable identification of pathogens of many infectious diseases.

The feasibility of using the immunofluorescence method for detection of F. tularensis has been demonstrated in a number of works, by both Soviet and foreign authors [1-5, 7, 10, 11, 14, 15, 17]. With this method, F. tularensis can be demonstrated in impression smears, histological sections of organs and tissues from animals infected with F. tularensis, smears of different dilutions of pure and mixed cultures, and in other preparations. The lowest concentration of F. tularensis consistently demonstrable by the immunofluorescence method is 1 million bacterial cells (according to the standard of the State Control Institute of Medical Biologicals imeni L. A. Tarasevich--GKI) per ml tested suspension. In lower concentrations, the pathogen is demonstrable but not in 100% of the cases. The sensitivity of this technique is close to that of the method of making cultures of animal organs on nutrient media [3].

Our objective here was to determine whether it is possible to use the immunofluorescence method for detection of F. tularensis in yolk sacs of developing chick embryos. The need for such studies is attributable to the fact that the usual staining methods according to Gram, Romanovsky-Giemsa and Zdrodovskiy are not very suitable for this purpose. This is related to the fact that F. tularensis does not have an elective color, whereas the presence in yolk sacs of significant amounts of lipids, phosphoproteins and pigment groups makes it difficult to differentiate between F. tularensis and tissular elements. Most researchers concerned with cultivation of F. tularensis in chick embryos assessed bacterial reproduction according to titration of a suspension of yolk sacs in mice or by inoculation on solid nutrient medium [13, 18]. These methods are rather sensitive, but they do not permit rapid determination of presence of F. tularensis in chick embryos.

Using the immunofluorescence method (we were unable to find any works dealing with use thereof for detection of F. tularensis in chick embryos in the literature available to us), we tried to identify the tissues and fluids, in which the pathogen of tularemia multiplies, track the dynamics of its accumulation as related to day of embryo death and magnitude of infective dose.

In our work, we used standard virulent strain No 503 (Holarctic race) and Schu (Nearctic race). We conducted the studies on developing chick embryos incubated for 8-10 days. Preliminary experiments revealed that embryos of this age are the most suitable model for cultivation of F. tularensis. Infection was produced in the cavity of the yolk sac by the conventional method [12]. For infection, we used bacterial suspensions prepared by suspending in saline 24-h cultures of F. tularensis grown on solid medium of fish hydrolysate with cystine, glucose and blood. The cultures were standardized to $10^9$ bacterial cells per mℓ (according to GKI standard). With the obtained suspension, we prepared successive 10-fold dilutions (from $10^9$ to 1 bacterial cell/mℓ). Each dilution, in a volume of 0.5 mℓ, was injected in 6-7 embryos. Concurrently, we checked the material for F. tularensis by means of culturing 0.1 mℓ of each dilution on a medium of fish hydrolysate with cystine, glucose and blood, or coagulated yolk medium.

After infection, the embryos were incubated at 37° at high relative humidity. The eggs were examined daily. Dead embryos were dissected the same day. We prepared impressions simultaneously on two thoroughly defatted slides, which were air-dried at room temperature, of pieces of yolk sacs slightly pressed down on filter paper. One of the impressions, without prior fixing, was stained with a mixture of Giemsa stain and methyl alcohol taken in equal parts [6, 9]. With this staining method, F. tularensis presented a violet color and could be clearly distinguished against a light lilac background. The other impression was fixed for 20 min in 96° ethyl alcohol, then stained with fluorescent equine tularemia serum (series No 859 produced by the Institute of Epidemiology and Microbiology imeni N. F. Gamaleya). For staining, we used the conventional direct method if fluorescent antibodies [2]. In order to "quench" nonspecific fluorescence, the preparations were treated with a mixture of fluorescent serum and bovine albumin labeled with rhodamine sulfofluoride, which were taken in 1 dilution lower than the working dilution, and applied to the preparations. Staining was continued for 20 min, after which the preparations were washed in 0.15 M NaCl for 10 min at a time, twice, changing the solution. Before examination, a drop of solution containing 1 part 0.15 M NaCl and 9 parts glycerin was applied to the preparation, and it was covered with a cover glass.

The preparations were examined in the incident light of an ML-2 fluorescence microscope using an immersion objective and system of light filters.

In positive cases, when the preparation contained F. tularensis, bacteria with specific bright green-yellow fluorescence were demonstrable on a dark or reddish (from rhodamine) background. Most often, there was bright fluorescence of the entire bacterial cell, rather than only the rim, as in most bacteria. This is related to the fact that F. tularensis has a mucous capsule-like sheath that adsorbs fluorescent antibodies on its surface, which prevents distinct visibility of the fluorescent rim and dark center of the cell. We failed to demonstrate any specific fluorescence in any impressions of yolk sacs from a normal chick embryo of the same age taken as a control.

A study by the immunofluorescence method of accumulation of F. tularensis in tissues and fluids of an embryo (9-days old) that perished on the 4th day after infection with $10^9$ bacterial cells of virulent strain No 503 revealed that a significant quantity of bacteria was contained in all fields of vision of the yolk sac impression smear, irregular amount of bacteria (ranging from considerable to isolated cells) in the chorioallantois membrane, isolated bacteria in allantois fluid and a significant number of bacteria in the extra-embryonic fluid after dissection of the yolk sac. Analogous findings had been reported by other researchers with regard to accumulation of F. tularensis in yolk sacs [13, 16].

Studies of the dynamics of accumulation of F. tularensis in yolk sacs as a function of time of death of embryos infected with virulent strain No 503 ($10^9$-$10^7$ bacterial cells) revealed the following: on the first day of deaths, bacteria were not demonstrable or else there were isolated bacteria in isolated fields in half the cases; on the 2d day, the quantity of bacteria increased, and they were demonstrable in all fields of vision, from isolated ones to a considerable number in different preparations. On the 3d day, we observed considerable quantities in all preparations. As a rule, the embryos did not survive to the 4th day. The surviving ones were dissected, and isolated bacteria were demonstrable in isolated fields. The latter is apparently related to individual embryo sensitivity to F. tularensis.

Results of using luminescence and light microscopy for demonstration of F. tularensis in chick embryo yolk sacs

| Strain | Infective dose (bacter. cells) | Embryo lethality (deaths/inf | Average day of embryo death | Culture |  | Microscopy results |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  |  |  |  |  |  | fluorescence (f) |  | light (l) |  | coincidence |  | divergence |
|  |  |  |  | Yolk medium | blood-fish agar | + | − | + | − | + | − | l+f− |
| Virulent strain No 503 | $10^9$ | 7/7 | 2,4 | + | + | 7 | 0 | 6 | 1 | 6 |  | 1 |
|  | $10^8$ | 7/7 | 3.1 | + | + | 7 | 0 | 7 | 0 | 7 |  |  |
|  | $10^7$ | 7/7 | 4.3 | + | + | 7 | 0 | 7 | 0 | 7 |  |  |
|  | $10^6$ | 7/7 | 4.0 | + | + | 6 | 1 | 6 | 1 | 6 | 1 |  |
|  | $10^5$ | 7/7 | 4.0 | + | + | 6 | 1 | 5 | 2 | 5 | 1 | 1 |
|  | $10^4$ | 7/7 | 4.6 | + | + | 7 | 0 | 7 | 0 | 7 |  |  |
|  | $10^3$ | 4/7 | 5.0 | + | + | 7 | 0 | 5 | 2 | 5 |  | 2 |
|  | $10^2$ | 4/6 | 6.0 | − | + | 6 | 0 | 2 | 4 | 2 |  | 4 |
|  | $10^1$ | 4/6 | 7.4 | − | + | 5 | 1 | 4 | 2 | 3 | 1 | 2 |
|  | 1 | 3/5 | 6/3 | − | − | 3 | 2 | 2 | 3 | 2 | 2 | 1 |
| Totals: absolute | | 57/66 |  |  |  | 61 | 5 | 51 | 15 | 50 | 5 | 11 |
|  | % |  |  |  |  | 92,3 | 7,7 | 77,3 | 22,7 | 83,3 |  | 16,7 |

Studies of dynamics of accumulation of F. tularensis in yolk sacs as a function of magnitude of infective dose revealed (see Table) that both the percentage and time of embryo death depended on the size of the infective dose. For example, with administration of $10^9$-$10^4$ bacterial cells, embryo death was observed in 100% of the cases on the 3d-4th postinfection day, whereas a dosage of $10^3$ or less elicited partial death on the 5th-7th postinfection day. Positive results for F. tularensis were observed in 92.3% of the cases according to fluorescence microscopy and 77.3% with light microscopy. The findings coincided in 83.3% of the cases and differed in 16.7% (positive results with fluorescence microscopy with negative data from light microscopy). There was particularly graphic manifestation of the advantage of the fluorescence method with use of doses of $10^3$ or less for infection, when positive results were noted in only 50% of the cases with light microscopy and 85% with fluorescence microscopy.

The specificity of the obtained data is also confirmed by the positive results we obtained when culturing material on yolk medium and medium of fish hydrolysate with cystine, glucose and blood. As a rule, the bacterial content thereof was lower by 1-2 dilutions than in embryos. The latter was indicative of reproduction of virulent strain No 503 in embryos.

An analogous pattern was observed with cultivation of the Schu strain in embryos. After giving $10^7$-$10^3$ bacterial cells, the embryos died on the 2d-3d postinfection day, with considerable accumulation of the pathogen. With use of $10^2$ bacterial cells or less, the embryos survived in a higher percentage of cases. Dissection of live embryos on the 8th postinfection day or later revealed isolated bacteria in the smears, in all or some fields of vision. Fluorescence of Schu bacteria was just as bright as that of strain No 503.

Use of the fluorescence and serological method for demonstration in yolk sacs of avirulent strain No 21/400 and attenuated strain No 503 failed to yield positive results. This is attributable to the fact that the specificity of fluorescence is due to interaction between labeled antiserum and Vi antigen substances of F. tularensis [8]. Consequently, with fluorescent antibodies obtained for the virulent strain, one can succeed in demonstrating virulent strains of F. tularensis, which have a rich antigen complex, or vaccinal strains, since they also contain a certain amount of Vi antigen. Completely avirulent or markedly attenuated strains deprived of the Vi antigen complex lose the capacity for specifically reacting with ordinary antitularemia fluorescent serum. Thus, the immunofluorescence method, which has high specificity and rather high sensitivity, with no equal in speed of obtaining results, can be recommended for demonstration of F. tularensis when examining tissues of chick embryos infected with the pathogen of tularemia.

## Conclusions

1. The immunofluorescence method can be recommended for detection of F. tularensis in chick embryo yolk sacs.

2. The immunofluorescence method is more sensitive than light microscopy; it permits detection of F. tularemia in smears of yolk sacs infected with minimum doses.

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# PHAGOCYTIC ACTIVITY OF BLOOD NEUTROPHILS IN THE PRESENCE OF TULAREMIA IN ANIMALS DIFFERING IN SENSITIVITY TO INFECTION

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[Article by T. N. Dunayeva and K. N. Shlygina, Institute of Epidemiology and Microbiology imeni Gamaleya, USSR Academy of Medical Sciences, Moscow]

[Text] In the case of tularemia, increased ingestive activity of blood neutrophils is observed in people and laboratory animals who have been inoculated or had this disease [1, 3, 4, 11]. Studies of tissue cultures revealed that there was an increase in digestive activity of peritoneal monocytes and polynuclear leukocytes in immune guinea pigs and rabbits [12, 13].

Our work involved a comparative study of ingestive and digestive capacity of polymorphonuclear leukocytes with reference to F. tularensis in animals differing in sensitivity to infection. We tested the opsonophagocytic reaction (OPR) of intact animals and animals infected in the course of the infectious process. We used the standard technique for the OPR: to a specific volume of citrated blood we added live virulent culture, obtaining 500 million bacteria/mℓ in the suspension to be tested. Fixed smears were stained according to Romanovsky-Giemsa diluting the stain in acetone [7]. We estimated the ingestion index of OPR in smears by the method that is used for brucellosis. We determined that phagocytosis was completed on the basis of the ratio of indexes found after 60 min and 2 h to the initial index, estimated after 30 min. An increase in quantity of bacteria in neutrophils, as well as increase in percentage of phagocytic cells, was indicative of mild digestive capacity of leukocytes [2]. We determined the intensity of OPR in intact animals: white mice, common voles, golden hamsters and guinea pigs, which are highly sensitive to tularemia, and albino rats and rabbits, which are not sensitive species.

Ingestion of F. tularensis by polymorphonuclear leukocytes was demonstrated in all animal species. After 30 min, the OPR index varied in different experiments, regardless of degree of sensitivity to tularemia infection of the animals (Table 1).

As shown by determination of digestive capacity of neutrophils, after 60 min, the OPR index exceeds the initial value by an average of 2 or more times in highly sensitive animals, and by 4-6 times in some white mice and guinea pigs. In some guinea pigs, the ingestion index rose insignificantly, while some animals showed a lower level than initially.

Table 1. OPR with F. tularensis in intact animals

| Animal species | Number of experim. | Number of animals | Ingestion index after |  |  |  | Index ratio (60/30) |
|---|---|---|---|---|---|---|---|
|  |  |  | 30 min |  | 60 min |  |  |
|  |  |  | range | mean | range | mean |  |
| White mice | 4 | 19 | 4—13,2 | 8,2 | 11,7—28,7 | 19,9 | 2,4 |
| Common voles | 2 | 8 | 4,5—6 | 5,0 | 14,3—16 | 15,1 | 2,8 |
| Golden hamsters | 1 | 3 | 3—6 | 4 | 14—16 | 15 | 3,7 |
| Guinea pigs | 5 | 19 | 3—12,8 | 7,7 | 11,6—25,6 | 17,2 | 2,2 |
| Albino rats | 3 | 12 | 6—21,3 | 11,3 | 15,2—21,6 | 18 | 1,5 |
| Rabbits | 7 | 19 | 4—20 | 12,4 | 4—25 | 13,8 | 1,1 |

In insensitive animals, the ingestion index increased to a lesser extent after 60 min than in highly sensitive ones. Many rabbits presented a marked digestive capacity of leukocytes. Mean ratios of indexes in different experiments ranged from 0.5 to 2.1 in rabbits and 1.1 to 2.8 in albino rats.

After 30 min, the quantity of phagocytic cells ranged from 16 to 53% in white mice, 13 to 51% in guinea pigs, 24 to 30% in white rats and 16 to 68% in rabbits. After 60 min, there was an increase in number of active neutrophils in all highly sensitive animals and albino rats. In some rabbits, the percentage of phagocytic cells diminished, or the increase was insignificant, which was indicative of manifestation of a bactericidal or bacteriostatic effect.

Changes in OPR were tracked on 30 white mice and 16 rats infected hypodermically with virulent strain No 503 in a dosage of 10 bacterial cells according to the optical cloudiness standard. At each time, we tested 4 animals. White mice were sacrificed; we took blood in vivo from the tail or heart of the albino rats. Reactions were run simultaneously on all animals tested from the 1st to 8th experimental days, and in rats on the 15th-20th postinfection day.

The ingestion index with 30-min exposure rose consistently in mice from the 2d to 6th day  Mice died on the 6th-7th day, whereas in rats the ingestion index decline on subsequent days and, on the 15th-20th day, did not differ from the index of intact animals. On the first postinfection day, there was minimal digestive function of neutrophils: in both mice and rats, the ingestion index was 2.6-2.9 times higher after 60 min than the initial level, which corresponded to the parameters of intact animals (Table 2). Thereafter, there was chronographic demonstration of an increase in ingestion index and percentage of phagocytic cells in mice. Consequently, phagocytosis had not terminated.

Upon examining blood smears of mice, we were impressed by localization of F. tularensis on small collections of thrombocytes. This interaction of blood platelets and bacteria increased from the 1st to 5th postinfection day and was minimal in intact mice. On the 6th day of disease, there was a reduction in accumulations of thrombocytes, and they virtually disappeared in some animals. At this time, the smears showed an increase in quantity of freely arranged bacteria, which were not fixed on thrombocytes or in a state of attraction to leukocytes. There were degenerative changes in neutrophils: vacuolization of protoplasm, pyknosis and phagolysis. Vacuoles with undigested bacteria were visible in destroyed cells. On the 4th-5th day of disease, plasmocytes and, occasionally, macrophages appeared in blood. Phagocytic monocytes were seldom encountered. Mice seldom presented agglomerates of neutrophils and lymphocytes against the background of accumulations of platelets and bacteria.

Unlike mice, the rats clearly showed greater completion of phagocytosis, according to both the index ratio and decline in percentage of phagocytic neutrophils with increase in reaction time (see Table 2). Already on the 2d postinfection day, OPR index after 60 min and 2 h were only negligibly higher than after 30 min. Thereafter, there was a consistent decline of the index, as well as quantity of phagocytic neutrophils with increase in reaction time. This intensification of digestive activity of neutrophils persisted over the entire observation period (up to the 20th day of the experiment), and it persisted even with decline of ingestion index. Thus, chronographic determination of the OPR index revealed qualitative new distinctions in correlations between phagocytic cells and the pathogen, which cannot be demonstrated by single determination of phagocytotic activity.

Microscopically, the reaction of rat blood elements differed from that of mice in that there were larger accumulations of F. tularensis on blood platelets, more frequent agglomerates of such collections with neutrophils and connected lymphocytes and monocytes. The latter phagocytized bacteria somewhat more often. The neutrophils showed distinct digestion of bacteria; some of them were faintly stained. There were many lysing leukocytes, and in the residues of destroyed cells there were digestive vacuoles, usually empty or containing insignificant amounts of bacteria.

Phagolysis of neutrophils in citrated blood with addition of bacteria causes extracellular death of bacteria as has been demonstrated, for example, with plague, by releasing bactericidal lisosomal proteins. Immunization increases lysability of neutrophils and release of lysosomes from them [6]. The decline of the ingestion index in our experiments coincided with appearance in rat blood serum of antibodies to F. tularensis in titers of 1:10-1:320, and change of infection to the extinction phase [10]. This shows that the decline of the index could be due to more intensive digestion of F. tularensis and faster phagolysis, rather than decline of ingestive activity of cells. At this time, infected rats showed a decrease in intensity of organ contamination with the pathogen and transition of infection to the extinction phase [10].

Unlike mice, rats showed neutrophils with degenerative changes only on the 3d postinfection day and only after 2-h contact with the pathogen. On subsequent days, along with significant phagolysis, the smears showed more neutrophils without degenerative changes in the nucleus, but they had large vacuoles with F. tularensis. On the 7th-8th day and later, the neutrophils reverted to normal, the vacuoles were small and there was well-marked granularity. At this time, the smears showed an appreciable decrease in quantity of F. tularensis, lying freely or bound with thrombocytes.

Table 2. Dynamics of OPR on different postinfection days

| Object examined | Reaction time (min) | Postinfection day |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  |  | 1 |  | 2 |  | 4 |  | 6 |  | 7 |  | 8 |  | 15 |  | 20 |  |
|  |  | ingest. index | active phago-cytes,% | ingest. index | active phag.,% | ingest. index | active phag.,% | ingest. index | active phag.,% | ingest. index | active phag.,% | ingest. index | active phag.,% | ingest. index | active phag.,% | ingest. index | active phag.,% |
| Infected mice | 30<br>60<br>120 | 3<br>8<br>12,5 | 12<br>32<br>46 | 5<br>12<br>17,7 | 20<br>49<br>71 | 8,7<br>9,7<br>19,2 | 35<br>39<br>77 | 14,2<br>26,5<br>22,2 | 67<br>83<br>89 | Died |  |  |  |  |  |  |  |
|  | Index ratio:<br>60/30<br>120/30 | 2,6<br>4,1 |  | 2,4<br>3,5 |  | 1,1<br>2,2 |  | 1,8<br>1,5 |  | — |  | — |  | — |  | — |  |
| Infected rats | 30<br>60<br>120 | 5,7<br>17<br>14,7 | 23<br>67<br>59 | 12<br>13<br>15,5 | 51<br>54<br>62 | 21,7<br>20,5<br>10,7 | 83<br>81<br>43 | 22,2<br>20,2<br>15,2 | 81<br>77<br>61 | 14<br>10<br>9,5 | 57<br>40<br>38 | 9,2<br>6,2<br>6,7 | 37<br>25<br>27 | 6<br>8,5<br>3,7 | 24<br>34<br>15 | 6<br>5<br>3,2 | 24<br>20<br>13 |
|  | Index ratio:<br>60/30<br>120/30 | 2,9<br>2,5 |  | 1,08<br>1,2 |  | 0,9<br>0,4 |  | 0,9<br>0,6 |  | 0,7<br>0,6 |  | 0,6<br>0,6 |  | 1,4<br>0,6 |  | 0,8<br>0,5 |  |
| Intact rats | 30<br>60<br>120 | 6<br>17,3<br>19 | 24<br>62<br>72 | — | — | — | — | — | — | — | — | — | — | 7,5<br>15,2<br>20,2 | 30<br>61<br>83 | — | — |
|  | Index ratio:<br>60/30<br>120/30 | 2,8<br>3,1 |  | — |  | — |  | — |  | — |  | — |  | 2,0<br>2,5 |  | — |  |

We observed adhesion of bacteria to thrombocytes in experimental animals by using the OPR with other pathogens--Listeria, Salmonella, pseudotuberculosis bacteria. This phenomenon was described in 1917 by Rickenberg (quoted in [8]) with reference to parasitic diseases. Subsequently, the reaction was also demonstrated in bacterial infections. Some researchers interpret clumping of blood platelets with bacteria as the first phase of phagocytosis [5]. According to current conceptions, aggregations of thrombocytes, bacteria and leukocytes are formed in the body under the influence of immune processes, with involvement of the antigen-antibody complex [9].

## Conclusions

1. The ingestive capacity of phagocytic cells of intact animals fluctuates over a wide range, without reflecting the level of sensitivity to tularemia infection.

2. In the course of development of infection, the ingestion index after 30-min exposure to the reaction increases in mice and rats, reaching a maximum on the 5th-6th postinfection day and ending with death of mice. In rats, the ingestion index declines after the 7th day of infection and corresponds to the index of intact animals on the 15th-20th day.

3. Substantial differences in digestive activity of mouse and rat neutrophils are demonstrable already on the 2d postinfection day. Phagocytosis is incomplete in mice, whereas in rats completion of phagocytosis and decrease in quantity of free bacteria in citrated blood are observed 4-20 days after infection.

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# NEW DATA ON INCIDENCE OF ENDEMIC INFECTIONS IN ARCTIC REGION OF EAST SIBERIA

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[Article by G. V. Kornilova, M. I. Raykhlin, V. K. Yastrebov, M. S. Shayman, L. S. Yegorova, F. F. Busygin, I. I. Bogdanov and N. A. Rogatykh, Omsk Institute of Endemic Infections]

[Text] Several biomedical problems arise in connection with the prospects of economic development of northern Siberia, among which investigation of the incidence of endemic diseases is of special importance. Studies of endemic sites for anthropozoonoses in regions of the Extreme North is a new and important aspect of development of teaching on endemicity of human diseases, which should elucidate the complicated aspects of the epidemiological process at high geographic latitudes, mechanism of formation and evolution of endemic sites and ecology of pathogens. These studies are also of definite practical value, primarily for planning and implementing measures for the prevention of endemic infections in the Arctic region.

In accordance with the plan for work on the problem of "Endemic Diseases of Man," for the purpose of detecting anthropozoonotic sites in the Extreme North (arbovirus infections, endemic rickettsiosis, tularemia, leptospirosis, toxoplasmosis), in 1972-1974, we conducted a combined epidemiological, virological, bacteriological and zooparasitological study on the territory of Taymyr National Okrug.

Of the above-mentioned infections only tularemia in this territory has been described in the literature [4], data being submitted on incidence of the disease among the inhabitants and isolation in the suburbs of Norilsk of the pathogen of infection from the root vole and Middendorff vole, whereas involvement of the typical tundra rodent species, lemmings (Lemmus Lemmus L.) in tularemia epizootics remained undetermined. For this reason, we formulated a specific task: to confirm bacteriologically tularemia epizootics among lemmings and, in this way, to obtain due data on endemicity of this infection. Concurrently, we investigated nest-burrow biocenoses in view of the possibility of having arboviruses carried in by migratory birds nesting in Taymyr and wintering in countries of the subequatorial, tropical and subtropical climate belts [5, 6].

In all, we submitted 1900 gamasid ticks from 77 nests of Siberian lemmings to virological examination, 398 spleens from lemmings and Middendorff voles to bacteriological examination, 1064 blood serum samples from the indigenous inhabitants, 1990 from reindeer, 466 from lemmings and 113 from Arctic foxes in captivity ["kept in cages"] to serological testing.

The results of serological studies using the hemagglutination extinction reaction (HAER) with arbovirus antigens revealed that antibodies were present to viruses of the tick-borne encephalitis complex and West Nile fever among the indigenous population who had never left Taymyr (Table 1), as well as the reindeer. Positive responses were obtained in several different places located both in the western and eastern parts of the peninsula, which was indicative of viral activity over an extensive territory, rather than specific sites, i.e., probability of widespread sources of infection in nature (the studies were conducted in 9 populated centers).

Table 1. Results of serological tests on indigenous population (aborigenes) and reindeer of Taymyr Peninsula for demonstration of tick-borne encephalitis and West Nile viruses

| Region of study | Object tested | Number of serum samples | Demonstration of antibodies to virus, % (M±m) | | Reciprocals of titers of antibodies to virus | |
|---|---|---|---|---|---|---|
| | | | TBE | WNF | TBE | WNF |
| Western Taymyr | Indigenous population | 367 | 2.2±0.7 | 0.8±0.4 | 10-40 | 10-80 |
| | Reindeer | 1088 | 0.7±0.2 | 0.7±0.2 | 10-80 | 10-160 |
| Eastern Taymyr | Indigenous population | 274 | 1.1±0.6 | - | 10-40 | - |

Key: TBE) tick-borne encephalitis WNF) West Nile fever

In the tundra regions, lemmings could play a part in the widespread sources of infection, since bloodsucking arthropods inhabit their nests, among which there is prevalence, according to our data, of parasitic gamasid ticks, Haemogamasus ambulans Thorel. and Hirstionyssus isabellinus Oudms.(over 70%). In the absence of ixodes ticks, it should be assumed that virtually the only probable factor in transmission of arboviruses to man and reindeer, with positive serological tests, could be mosquitoes.

The nests of lemmings were collected for examination in August 1973, in the vicinity of the Novorybnaya trading post in Khatangskiy Rayon, on the right bank of the Khatanga River (73-74° north latitude). We extracted gamasid ticks from the nest using electric "thermoeclectors," 41% of them being referable to Hg. ambulans and 32.6% to Hi. isabellinus.

From a sample containing 836 Hg. ambulans and Hi. isabellinus gamasid ticks, used to infect guinea pigs intraperitoneally with 10% suspension thereof in 1973, a strain of tick-borne encephalitis was isolated.*

Isolation of the virus of tick-borne encephalitis in the subarctic tundra was an utterly new finding, which indicated that, in the absence of ixodes ticks

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*Additional identification was made at the Institute of Virology imeni Ivanovskiy, USSR Academy of Medical Sciences.

Table . Incidence of demonstration of antibodies to pathogens of leptospirosis, endemic rickettsiosis and toxoplasmosis among indigenous inhabitants and some animals on Taymyr peninsula in 1972-1974 (M±m)

| Object examined | To leptospirosis virus |  | To Asian tick-b. encephalitis virus |  | Q fever |  | Toxoplasmosis |  |  |
|---|---|---|---|---|---|---|---|---|---|
|  | serum samples tested | incid. of antibod, | serum samples tested | incid. of antibod. | serum samples tested | incid. of antibod. | serum samples tested | incidence of antibody demonstration |  |
|  |  |  |  |  |  |  |  | CFT | IF |
| Indigenous population | 945 | 10,4±1,2 | 1064 | 2,1±0,4 | 1064 | 1,3±0,3 | 584 | 5,5±0,9 | 9,8±1,4 |
| Reindeer | 1698 | 11,6±0,9 | 1990 | 2,6±0,4 | 1990 | 1,3±0,3 | 650 | 4,1±0,8 | — |
| Lemmings | 397 | 0,7±0,2 | 406 | 4,1±0,9 | 466 | 1,7±0,6 | 58 | — | 13,8±4,5 |
| Arctic foxes in captivity | 113 | 16,8±4,3 | 69 | 3,0±0,9 | 69 | — | 113 | — | 7,1±4,5 |

Note: Serological testing for leptospirosis was performed using the lysis agglutination reaction, for rickettsiosis using the CFT [complement fixation test], for toxoplasmosis using the CFT and immunofluorescence (IF) test.

there could be arbovirus sites in the nest-burrow biocenoses of lemmings. The obtained data were also interesting in that heretofore the existence of arbovirus sites in the Extreme North was known only in biocenoses of bird rookeries, where tick-borne encephalitis virus was isolated in the northern part of European USSR, the Far East and United States [1], as well as arboviruses new to science--Tyuleniy, Okhotskiy, Sakhalin and others [2, 3, 7, 8]--from Ixodes ticks (Ceratixodes putus Pick-Cambr, which is a parasite of aquatic birds, mainly guillemots [or murres]).

Endemic sites for tularemia in Taymyr were studied after preliminary serological testing. In 1972, when the lemming population was small, we obtained positive reactions with tularemia antigen in the indirect hemagglutination test in 5 out of 94 animals collected along the bank of the Pyasina River (West Taymyr). This could be indicative of the probability of local scattered epizootic outbreaks of tularemia among lemmings in a period of depression in their number. In 1973, the lemming population grew drastically, and isolated lemming carcasses were found in the vicinity of the Novorybnaya trading post, which was indicative of an incipient epizootic. Bacteriological examination of lemming spleens resulted in isolation and identification of six cultures of F. tularensis. Thus, there was bacteriological confirmation of the existence in Taymyr of endemic tundra (lemming) sites of tularemia. Full-fledged epizootics involving large territories were observed during periods of mass reproduction of lemmings. Since this type of tularemia site had not been examined in Taymyr, investigation thereof at the present time should be viewed as one of the first and foremost tasks in the area of regional pathology.

In 1974, there was a severe depression of the lemming population throughout Taymyr, for which reason we conducted studies to determine whether the pathogen of tularemia could be retained in the environment. We submitted to bacteriological examination 60 samples of moist substrate in the vicinity of nests, which had been gathered from the same lake-swamp region of the tundra. Cultures of F. tularensis were isolated from 2 samples of moist substrate. Studies of morphological and cultural properties, as well as virulence of the cultures, were indicative of their belonging to the Holarctic race. Examination of washings from 9 Middendorff vole nests revealed a culture of Listeria in one of them (in the vicinity of the Kresta trading post, 72° north latitude).

The results of serological tests on the public and animals for leptospirosis indicated (Table 2) that the etiological structure of these diseases among the inhabitants and reindeer virtually coincided, and that it was characterized by marked prevalence of the Australis and Ballum serogroups. Evidently, adaptation of Leptospira to arctic conditions is associated with elimination of certain serogroups that prevail at moderate latitudes of Siberia. Nor can we rule out the existence of lemming sites of leptospirosis, as indicated by serological data obtained in Taymyr. However, the incidence of seropositive results in tests with Leptospira referable to different serological groups is 1/12th-1/18th in lemmings of the level in humans and reindeer.

Together with veterinary workers, we found an outbreak of leptospirosis among blue Arctic foxes of the animal farm at the Sovetskiy Taymyr kolkhoz (72° north latitude). In the summer of 1973, fox deaths were observed on this farm. The leptospirous etiology of animal diseases was confirmed by pathological and serological studies. Antibodies to Leptospira, of the Grippotyphosa, Bataviae, Pomona and Ballum serogroups (15.8%), were demonstrated, and Grippotyphosa and Bataviae accounted for over 60% of the seropositive findings. No more deaths occurred after the foxes were immunized with polyvalent serum against leptospirosis of farm animals.

The positive results of serological tests on indigenous inhabitants, reindeer and lemmings for tick-borne Asian rickettsiosis and Q fever (see Table 2) were indicative of circulation of the pathogens of these infections in the Arctic.

Antitoxoplasma antibodies were demonstrated not only in the CFT, but by the immunofluorescence method. We should mention the relatively high titers of serological reactions (1:10-1:20) in one-third of the cases. In view of the nonsterile nature of immunity to toxoplasmosis, as well as data indicative of specificity and sensitivity of the serological reactions we used, we believe that toxoplasmosis is rather widespread in the northern part of East Taymyr. We demonstrated for the first time that the population of the Taymyr National Okrug was stricken by toxoplasmosis. Since there were virtually no house cats in most of the villages we screened, and they are the final hosts of Toxoplasma, reindeer could be a possible source of this infection in man, and 4.1% of them were found to be stricken in this region.

Thus, as a result of our combined studies of anthropozoonotic sites in the subarctic tundra of Taymyr, the existence of tick-borne encephalitis virus was demonstrated in the nest-burrow biocenoses of lemmings, as well as the existence of infection with F. tularensis in this typical and widespread rodent of the tundra. At the same time, data were obtained on the presence of combined sites of these two infections, which are ecologically linked to lemmings, at high latitudes. The appearance of sites of tick-borne encephalitis virus in Taymyr could be related to its being brought in by migratory birds. In addition, we should mention the ecological flexibility of pathogens of anthropozoonoses and the theoretical possibility of their adaptation to conditions in the Arctic where, with all the paucity of fauna, there is retention of species of vertebrates and arthropods that are close to the known hosts and vectors of these infections.

Conclusions

1. For the first time, in 1973, tick-borne encephalitis virus was isolated from Haemogamasus ambulans and Hirstionyssus isabellinus gamasid ticks beyond the range of their habitat, in the subarctic tundra on Taymyr Peninsula out of nests of the Siberian lemming, and antihemagglutinins to this virus and West Nile fever virus were demonstrated in the indigenous population and reindeer.

2. In the same location in Taymyr, cultures of F. tularensis were isolated for the first time from lemmings, which proves the existence of northern tundra sites of tularemia in the Arctic and also confirms the etiology of the tularemia epizootic among lemmings, which ocurred in 1973; in the period between epizootics, when there was a drastic depression of the lemming population and none at all in the region of our study, the pathogen of tularemia persisted in the tundra, in the environment.

3. Listeria was isolated from nests of Middendorff voles, which was indicative of the fact that this species of small mammals is stricken by the pathogen of listeriosis.

4. The results of serological tests on the inhabitants and animals were indicative of circulation of pathogens of leptospirosis, toxoplasmosis and endemic rickettsiosis in the arctic region of Eastern Siberia.

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# CULTIVATION OF F. TULARENSIS IN DEVELOPING CHICK EMBRYOS

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[Article by R. I. Kudelina and V. L. Popov, Institute of Epidemiology and Microbiology imeni Gamaleya, USSR Academy of Medical Sciences, Moscow]

[Text] The studies of a number of authors [1, 6, 8, 9, 12] have shown that developing chick embryos are highly sensitive to the pathogen of tularemia infection. This pathogen, when injected in the chorioallantois or cavity of the yolk sac, multiplies intensively in ectodermal and mesodermal cells of membranes, and it causes a lethal disease in the embryo. The authors determined development of tularemia infection on the basis of time of embryo death, pathological findings, as well as titration of a suspension of membranes and embryo tissues on glucose-cystine blood agar or white mice. The authors did not pursue morphological studies for demonstration of the pathogen by light or electron microscopy, with the exception of Larson [9], who used the Wayson method to stain smears. Unfortunately, he does not submit the results of these studies, so that we cannot evaluate the suitability of this staining method for demonstration of F. tularensis in embryos.

The absence of studies of this aspect is attributable to the fact that, because of an underdeveloped mucopeptide layer, F. tularensis has a thin and brittle [delicate] cell wall, which is difficult to stain and readily injured during various manipulations. The presence in yolk mass of significant amounts of lipids, phosphoproteins and pigment groups makes it difficult to differentiate between F. tularensis and various cellular inclusions. For this reason, the usual staining methods are unsuitable for detection of F. tularensis in chick embryo yolk sacs.

We investigated the possibility of using developing chick embryos for cultivation of both virulent and avirulent strains of F. tularensis. We used light and electron microscopy for detection of F. tularensis in embryos. We devoted special attention to determination of possibly different F. tularensis with respect to supramolecular organization, depending on virulence of the strains.

The studies were performed on 2000 chick embryos after 8-10 days of incubation. We failed to demonstrate any differences in sensitivity to infection in embryos of this age. Embryos incubated for 5-7 days were found to be less resistant and perished within 24-84 h after infection. Embryos incubated for 12-15 days were also unsuitable for experiments, since the time of their death due to infection with vaccinal or attenuated strains could coincide with the time of death occasionally observed in the period preceding hatching, which makes it difficult to interpret results.

The conventional Cox method [7] was used to infect the embryos in the yolk sac. For infection, we used strains of different geographic races and with different virulence: Schu (virulent), No 38 attenuated (Nearctic race), No 503 virulent, No 503 attenuated, No 15/10 vaccinal, No 21/400 entirely avirulent (Holarctic race). All of the strains were typical in morphological cultural and biochemical properties. In the experiments, we used day-old cultures of F. tularensis grown on solid agar medium of fresh fish hydrolysate with cystine, glucose and blood. The cultures were suspended in saline to $10^9$ bacterial cells/mℓ (by the standard of the State Institute of Standardization and Control of Medical Biologicals imeni Tarasevich); from the obtained suspension we then made 10-fold dilutions. Each dilution, in a volume of 0.5 mℓ, was injected in the yolk sac of 5-6 embryos, 0.1 mℓ was plated on agar medium of the same composition for demonstration of F. tularensis and extraneous flora. As a control, we used embryos of the same age in whose yolk sac 0.5 mℓ saline was injected instead of culture. The embryos were incubated at 37° and high relative humidity, which was produced by placing a tray with water in the incubator. The eggs were examined daily to determine time of embryo death. Dead embryos were dissected on the same day. We used 10% yolk sac suspension in saline for serial passages; it was injected in amounts of 0.5 mℓ in the yolk sac of the next batch of embryos.

Impressions were made of pieces of yolk sacs from dead embryos, which were air-dried, then stained with a mixture of Giemsa stain and methyl alcohol taken in equal parts [2, 3]. With this staining method, F. tularensis turned violet and cellular elements a light lilac. The advantage of this method over the usual Romanovsky-Giemsa staining method is that it is fast, simple and there is no preliminary fixation, as well as that it differentiates between bacteria and cellular inclusions.

The stained smears were viewed under a light microscope. Accumulation of bacteria was evaluated on a 4-point system: isolated--up to 10 bacteria (-), 10-20 bacteria (+), 50-100 (++) and over 100 per field (+++). To calculate the specific accumulation of bacteria, we arbitrarily took a parameter of 0.5 for presence of isolated bacteria, 1 for + accumulation, 2 for ++ and 3 for +++; we divided the total number by the number of embryos that died, in which F. tularensis was found.

For electron microscopy, we usually dissected live or agonal embryos, and in only some cases some that had died. Pieces of yolk sac membranes from chick embryos infected with F. tularensis were fixed in cold 1% glutaric aldehyde on phosphate buffer at pH 7.2 for 20-30 min. The material was then additionally fixed in 1% $OsO_4$ on phosphate buffer for 2 h at room temperature, with contrasting in 3% uranyl acetate and 30% ethanol for stabilization of bacterial nucleoid [9]; dehydration was performed in ascending concentrations of ethanol and absolute acetone; it was soaked in mixtures of acetone and epoxy resin and imbedded in araldite M [10] or a mixture of epon and araldite [11]. Ultrafine sections were made with an LKB-4800 ultramicrotome and contrasted with 5% aqueous solution of uranyl acetate. We used IEM -7 and IEM-100B microscopes with instrument magnification of 3000, 30,000 and 50,000×.

Examination of the cultural distinctions of F. tularemia in chick embryos revealed that virulent strains, Schu and No 503, multiplied intensively in yolk sacs causing embyro death on the 3d-4th postinfection days, even with administration of $10^{1}-10^{2}$ bacterial cells; vaccinal strain No 15/10 caused embryo death when given in a dosage of $10^{7}-10^{6}$, whereas with use of attenuated strain No 503 and completely avirulent strain No 21/400 embryo death occurred only with $10^{9}-10^{8}$ bacterial cells.

We demonstrated some correlation between virulence of strains and average time of embryo death (see Table). Thus, with administration of large doses of pathogen ($10^{7}-10^{9}$) average time of embryo death after infection with virulent strains Schu and No 503 was at 3.1-3.5 days, and with strain No 21/400 6.2 days. The percentage of embryos with positive bacterioscopic results, as well as the coefficient of specific accumulation of bacteria, were also considerably higher in embryos infected with virulent strains, as compared to avirulent ones (86-82.9% and 1.5 in the former case, 35.4% and 0.7 in the latter).

Results of cultivating F. tularensis in chick embryos

| Geographic race | Strain | Number of embryos infected | Embryo deaths[1] | Bacterio-scopically positive tests[2] | Specific accumula-tion of bacteria | Average time of embryo death (days) |
|---|---|---|---|---|---|---|
| Nearctic | Schu | 71 | 71/100 | 60/86 | 1,3 | 3,1 |
| Holarctic | No 503 (virulent) | 264 | 263/99,7 | 23[illegible]/82.9 | 1,8 | 3,5 |
| | No 15/10 (vaccinal) | 121 | 115/95 | 114/70 | 1,2 | 4,0 |
| | No 503 (attenuated) | 255 | 231/98,4 | 120/47,8 | 0,9 | 5,1 |
| | No 21/400 | 271 | 254/91,8 | 90/35,4 | 0,7 | 6,2 |
| | Totals | 982 | 954 | | | |

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[1] Denominator--% deaths in relation to number of infected embryos.
[2] Denominator--% deaths with positive bacterioscopic results.

Note: Infective dose was $10^{7}-10^{10}$ bacterial cells.

[illegible] of cultivation of strain No 38 are not listed in the table, since the embryos survived after being infected with these strains in a dosage of $10^{7}$ bacterial cells and there was a low percentage of deaths within the first [illegible] days with a dosage of $10^{8}$ bacterial cells. We failed to demonstrate F. tularensis in impression smears of pieces of yolk sac membranes from both dead and live embryos dissected on the 6th-12th postinfection day. We failed in our effort to adapt this strain to chick embryos by means of two blind passages:

bacterioscopy was negative, and no growth was demonstrable when a suspension of yolk sacs was plated on solid nutrient medium of fish hydrolysate with cystine, glucose and blood.

It was interesting to find out whether F. tularensis multiplies in chick embryos or is merely preserved there. To answer this question, we made a quantitative assay of F. tularensis in chick embryo yolk sacs. For this purpose, we prepared 10-fold dilutions from a suspension of yolk sacs (1 g/mℓ saline) and 0.1 mℓ of each dilution was cultured on medium of fish hydrolysate with cystine, glucose and blood. As shown by a comparison of culturing results to magnitude of infecting dosage, there was intensive reproduction of the pathogen of tularemia after infection with virulent strain No 503: there were several billion bacteria per gram yolk sac in an embryo infected with $10^7$ bacterial cells, which died on the 3d day. Attenuated strain No 503 did not multiply as profusely as the virulent one: there were $10^5$ bacterial cells per gram yolk sac. Strain No 21/400 did not multiply, but was merely preserved in the embryos: there were about $10^3$ bacterial cells per gram yolk sac, and when this strain was submitted to passages in embryos there was no increase in number of bacteria, on the contrary, it decreased and by the 3d passage no bacteria were demonstrable in the smears.

Examination of ultrafine sections of yolk sac membranes from embryos infected with F. tularensis revealed that bacterial cells usually presented extracellular localization. Occasionally, we find cells among the cellular debris that were invested in an additional three-layer membrane, apparently the membrane of an intracellular vacuole (see Figure, *c* [photo not reproduced]). On this basis, it can be assumed that the bacteria may also be situated within the epithelial cells of the yolk sac membrane, in cytoplasmic vacuoles.

As a rule, the cells of virulent strain No 503 were surrounded by a light [or clear] zone, apparently formed as a result of interaction between the mucoid bacterial sheath and imbedding medium. Such a zone was seen much less often around cells of other strains.

Variation of dimensions was inherent in bacteria of all tested strains: along with cells of the usual size for a given strain, we encountered larger ones in the population; this was particularly typical of strain No 21/400. F. tularensis cells were round or oval, with the following ranges of size: 500-700×400-600 nm (virulent strain No 503), 400-600×300-400 nm (attenuated strain No 503) and 500-800×300-700 nm (strain 21/400). The cells were invested in a cell wall with smooth surfaces. The three-layer membrane of the cell wall 60-90 A in thickness presented a symmetrical profile. In cells of avirulent strain No 21/400 and attenuated strain No 503, we occasionally demonstrated a more dense external layer of cell wall membrane, which was somewhat thicker, perhaps due to close adherence of microcapsular material. There were clusters of capsular material on the surface of such cells (see Figure, *б*). The inner layer of the cell wall had moderate electron density, its thickness ranged from 2-3 to 5-7 nm in some places. The cytoplasmic membrane presented the usual three-layer profile for Gram-negative bacteria, and thickness of 6-7 nm. A large nucleoid, which is inherent in these bacteria [4], occupied the center of the bacterial cell; the cytoplasm of moderate electron density occupied the periphery of the cell. Ribosomes 15-20 nm in diameter were demonstrable in the cytoplasm.

we failed to demonstrate basic difference in ultrastructure of virulent and avirulent strains of F. tularensis in chick embryo yolk sacs.

In noting the high sensitivity of chick embryos to tularemia infection, we must also mention the negative aspects of this method. In the first place, it is not standard, apparently due to the dissimilar resistance of embryos to infection, as a result of which some embryos die within the first postinfection days and others at a later time, in spite of being given the same infective dosage. We observed 100% embryo death only when infected with large doses. In the second place, the time of embryo death depends on the diet of fowl that produced them. As we know, some antibiotics, for example, tetracycline and grisin [derived from Actinomyces griseus], are used as biogenic stimulators and added to fowl feed. Traces of these agents in embryos could have an adverse effect on efforts to adapt F. tularensis to embryos, since this pathogen is highly sensitive to them. Yet, it is not deemed possible to determine with reliability whether fowl were on an antibiotic diet, since we generally used embryos referable to different batches, sometimes obtained from different poultry farms. The alternation, "without cause," of periods of profuse accumulation of F. tularensis and periods of minimal accumulation, which was unrelated to season and other factors, is indicative of the existence of such a diet. This must be taken into consideration when culturing F. tularensis in embryos.

Thus, the results we obtained from cultivating F. tularensis in developing chick embryos revealed that this biological model can be used to grow the pathogen of tularemia. It was learned that virulent strains multiplied, while attenuated and completely avirulent ones merely were preserved in chick embryos, and some, for example strain No 38, disappeared when passed in embryos. The difficulty of adaptation of strain No 38 to embryos is apparently related to its metabolic distinctions as a result of prolonged cultivation in U. S. laboratories on glucose and cystine blood agar. It should be noted that Downs et al. [8], also reported inconsistent results when this strain was cultivated in embryos. Embryo deaths were observed only with administration of 400 million bacterial cells, on the 15th-18th postinfection day.

In this case, use of a high-speed staining method using a mixture of Giemsa stain and methyl alcohol for demonstration of F. tularensis in smears of yolk sac membranes justified itself. This technique enabled us to record accumulation of the pathogen of tularemia without using the time-consuming process of counting colonies in dishes inoculated with different dilutions of yolk sac suspension.

The study of ultrafine structure of the pathogen of tularemia in chick embryo yolk sacs failed to demonstrate any basic differences between the structure of virulent and avirulent strains. When cultivated in this model, the structure of F. tularensis is similar to their ultrastructure when grown on solid nutrient medium [illegible].

## Conclusions

1. Chick embryos are highly sensitive to tularemia infection, and virulent strains of F. tularensis multiply intensively in chick embryo yolk sacs,

causing death thereof on the 3d-4th postinfection day, whereas virulent strains are merely preserved in them.

2. One can use a high-speed method of staining with a mixture of Giemsa stain and methyl alcohol or acetone for demonstration of F. tularensis in chick embryo yolk sacs.

3. We failed to demonstrate differences in ultrafine structure of virulent and avirulent strains of F. tularensis.

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# PRESERVATION OF F. TULARENSIS IN THE ENVIRONMENT IN EASTERN TAYMYR

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[Article by I. P. Algazin, V. A. Il'in and L. S. Yegorova, Omsk Institute of Endemic Infections]

[Text] Investigation of the possibility of existence and wide distribution of a number of endemic diseases, including tularemia, merits special attention in view of the ever increasing economic development of some regions in the Extreme North. There are sparse data in the literature concerning tularemia in the Arctic, and they pertain essentially to detection of this infection in the northern part of European USSR (Kola Peninsula, Arkhangelsk Oblast) [5-7]. The tundra zone of East Taymyr has been the least studied in this respect, although conditions exist there for existence of active endemic tularemia sites [1].

In 1973, we demonstrated, for the first time in the USSR, spontaneous infection of Siberian lemmings with F. tularemia in this region. Studies conducted the following year coincided with a severe depression of the population of this species--not a single lemming was trapped in natural biotopes in July-August 1974. For this reason, we concentrated mainly on a search for sites where the pathogen is preserved in nature. In view of the complete absence of Ixodes ticks in the Taymyr fauna, we assumed that F. tularensis could be preserved in water, the substrate of unoccupied nests and gamasid mites [ticks] that inhabit these nests during a period of lemming depression. The possibility of prolonged survival of F. tularensis in the environment was the subject of investigation by many Soviet and foreign researchers [2, 3, 8, 9]. It was established, in the field and experimentally, that low temperature, absence of direct insolation and high ambient humidity are the chief factors that are instrumental in preservation of this pathogen [2, 3]. The distinctions of the tundra landscape (small lakes formed in permafrost craters, numerous swamps, the water of which is in contact with the permafrost layer that is in the immediate vicinity of the ground cover) provide, in our opinion, optimum conditions in this respect.

We gathered material for bacteriological testing in the vicinity of the village of Novorybnoye (Khatangskiy Rayon of Taymyr National Okrug), in a region of moss-lichen tundra, at permanent observation posts. We collected water samples for analysis from exposed sources--glacier run-off, brooks, tundra lakes (7 samples) and moist substrate in the vicinity of nests in the summer habitats of lemmings (60 samples) which, according to absence of fresh traces of vital functions, had not been visited by the animals since the previous fall. Water samples were put in sterile vials, 150-250 mℓ in each. The method of collecting samples of the substrate near nests consisted of the following: we took the nest from a lemming burrow, then carefully collected the moist layer of soil around it, squeezed it over a vial and used the obtained suspension for biological tests. We took one water sample from each substrate. The nests gathered from burrows (63 nests) were also subsequently examined for tularemia. The obtained material was delivered to a field laboratory, where 0.5 mℓ of tested water was given by hypodermic injection under sterile conditions (in the right inguinal region) to white mice (2 mice per sample). Animals infected with each specimen were kept separately. We used a vaseline and paraffin mixture [4] to preserve first-passage viscera. Subsequent examination was made in the laboratory of the Omsk Institute of Endemic Infections. The animal organs were submitted to a second passage on white mice. We used coagulated yolk medium for demonstration of F. tularensis and beef-extract agar as a control. We identified the isolated strains using the complete agglutination test with specific tularemia serum (series No 17, titer 1:3200, shelf life up to November 1975).

In the first passage, 7 mice died on the 7th-8th day. Necropsy revealed an infiltrate at the injection site and considerable enlargement of the spleen in all cases. In addition to bacillary forms, we examined coccus bacteria suspect for tularemia in impression smears of viscera from three mice. The other animals were sacrificed after 10 days. We examined the spleen of dead (individually) and sacrificed (5-10 at a time) animals a second time by the biological method.

We succeeded in isolating two cultures of F. tularensis as a result of a second passage of white mouse organs infected with water from two different samples of substrate near nests. It must be noted that the cultures were isolated in a test of material from one of the regions where spontaneous tularemia infection had been demonstrated in 1973 among Siberian lemmings. The distance between sites of collection of water samples from the substrate near nests constituted about 100-150 m.

Death of second-passage animals infected with water samples occurred on the 5th-7th day. Necropsy revealed typical changes in internal organs: enlargement and hyperemia of vessels in the subcutaneous cellular tissue, enlargement and consolidation of the spleen and liver. Impression smears showed the morphological forms that were typical of F. tularensis. Typical mucoid ("pebbled leather" type) growth, which did not differ in color from the nutrient medium, was observed when lymph nodes, spleen, liver and blood were cultured on yolk medium, after 24-h incubation at 37°. There was no growth on agar. In smears of cultures submitted to Gram staining, we demonstrated Gram-negative coccobacteria. Both cultures were agglutianted to titer by diagnostic serum. The virulent properties of the isolated strains of F. tularensis did not differ from the strains isolated from Siberian lemmings in 1973: the completely lethal dose constituted 1 bacterial cell for white mice and 1 billion bacterial cells for albino rats (according to the standard of the State Institute of Standardization and Control of Medical Biologicals imeni Tarasevich).

Biological testing of washings from nests gathered in lemming burrows failed to yield positive results.

In view of these data, it is important to continue with investigations of the role of abiotic factors in preservation and distribution of tularemia, as it applies to the specific conditions of endemic sites in the Arctic).

Conclusions

1. The possibility of preservation of F. tularensis in the moist substrate near nests in summer habitats of lemmings during a period of depression in their number was demonstrated for the first time for high latitudes (73°north latitude).

2. The biological properties were retained, including virulence, in strains that were found under natural conditions in the near-nest substrate.

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# ESTABLISHMENT OF ENDEMICITY OF TULAREMIA IN TUVA ASSR

Moscow ZHURNAL MIKROBIOLOGII, EPIDEMIOLOGII I IMMUNOBIOLOGII in Russian No 2, Feb 76 (manuscript received 17 Apr 75) pp 75-78

[Article by M. I. Antsiferov, N. A. Zykina, L. V. Sizykh, T. G. Charnaya, V. R. Zharov, N. I. Yel'shanskaya and A. V. Yevdokimov, Irkutsk Scientific Research Plague-Control Institute of Siberia and the Far East, Tuva Republic Sanitary and Epidemiological Station, Tuva Plague-Control Station]

[Text] Tularemia had not been detected in Tuva ASSR up to 1974. However, the existence of endemic sites of this infection in neighboring republics, krays and oblasts (Buryat ASSR, Altay and Krasnoyarsk Krays, Kemerovo and Irkutsk Oblasts) warranted the belief that tularemia did exist in Tuva.

Examination of field material (rodents, ectoparasites, including Ixodes ticks, flying bloodsucking insects) for tularemia, which was conducted in 1968 in the laboratories of the Tuva Plague-Control Station and Department of Particularly Dangerous Infections of the Tuva Sanitary and Epidemiological Station was not so significant in volume, and the negative results it yielded could not warrant the conclusion that there were no endemic tularemia sites on the territory of Tuva ASSR.

It is now time to obtain a definitive answer to the question of presence or absence of endemic sites for tularemia in Tuva and, institute preventive measures on this basis in view of the rapid economic development and influx of people to this republic.

In 1974, the Irkutsk Plague-Control Institute, together with the Tuva Plague-Control Station and Department of Particularly Dangerous Infections of the sanitary and epidemiological station, conducted a survey to answer this question. Attention was focused mainly on gathering Ixodes ticks and submitting them to bacteriological examination.

A total of 20,099 Ixodes ticks were gathered between 16 and 29 April 1974 at 7 points in 4 rayons of this republic (Tandinskiy, Ulug-Khemskiy, Mongun-Tayginskiy, suburbs of Kyzyl). The main collections of ticks were made in the vicinity of Lake Khadyn (Tandinskiy Rayon) in a narrow coastal strip, where there is prevalence of Siberian pea-shrubs [Caragana arborescens Lam], water sedge short-grass meadows and chee grass. In this strip, there are numerous settlements in strips [bands] of the narrow-skulled vole, active burrows of the Daurian pika [Ochotona daurica Pallas] are common; it is also inhabited by the midday gerbil and long-tailed Siberian suslik. Lake Khadyn is used to water cattle and sheep. There, the number of ticks constituted about 300 per flag-h (flag 1×1.2 m in size made of flannel).* All of the collected ticks were referable to the same species, Dermacentor nuttalli.

The ticks were examined by the biotest method on white mice in the Tularemia Department of Irkutsk Plague-Control Institute (7011 specimens) and in the laboratory of the Tuva Plague-Control Station (13,088 specimens).

Bacteriological testing of 7011 ticks resulted in isolation of 11 strains of F. tularensis. Examination of 13,088 ticks yielded negative results. Ten strains were isolated from ticks gathered in the vicinity of Lake Khadyn and 1 from ticks (247) removed from cows at the Ulug-Khaya Sovkhoz in Ulug-Khemskiy Rayon. In addition, 4 strains were isolated somewhat later from ticks (5144) collected in the same rayon (by G. S. Letov) and tested in the laboratory of the Department of Particularly Dangerous Infections of the Tuva Republic Sanitary and Epidemiological Station. The territorial distance between the points where tularemia-infected ticks were found leads to the conclusion that there are scattered endemic sites of this infection in this republic.

Biotested mice died of tularemia on the 7th (1), 8th (2), 9th (3), 10th (1), 12th (2), 14th (2) and 19th (1) days, presenting the typical pathoanatomical signs of this infection. Liver and spleen F. tularensis content in these mice was given a score of 4.

Studies of morphological, cultural, tinctorial, serological and virulent properties confirmed that the isolated strains were referable to F. tularensis: bacteria did not grow on ordinary nutrient media; there was delicate, ciliated growth on yolk medium with no motility; all strains were agglutinated by specific serum in a titer of 1:3200 (1 to the serum titer and 1 to 1:6400). The virulence of all of the cultures was the same when determined in white mice--Dclm [clinically lethal median dose?] constituted 1 bacterial cell. Isolation of F. tularensis from ticks confirmed conclusively the presence of endemic sites of this infection in Tuva ASSR.

Another confirmation is referable to the results of our studies of retrospective detection of tularemia and a natural immune stratum among the population. Thus, in 1974, we found two people who had suffered from tularemia in 1972 and were infected in Tuva ASSR. They had been in the tayga, in the upper reaches of the Yenisey River from late August to 8 September, where they gathered field material for work on their diploma. They were infected by four muskrat carcasses, which the students found on lakes in the tayga and which they had skinned. Three days later, both students experienced malaise. This served as grounds to stop their work prematurely and leave the tayga to return to their homes (one in Irkutsk and the other in the village Kyren, Buryat ASSR). There, they became sick (on 8 and 10 September). Their illness was associated with high fever (for 6 days in one of them and 9 days in the other), headache, loss of appetite, severe weakness, nocturnal perspiration and axillary bubos. One of the patients spent 2 weeks in a hospital in the region of his residence with the diagnosis of axiallary lymphadenitis, the other stayed at home and did not seek medical attention. The result of the epicutaneous allergy test with tularin, which we did for retrospective confirmation of tularemia, was positive for the student who lived in Irkuts (infiltrate 2×2 cm in size). The expanded agglutination test for tularemia was positive in a titer of 1:100.

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*Zoologists from the Tuva Plague-Control Station participated in gathering ticks: G. K. Yevteyev, V. K. Melkova, Yu. V. Nikiforov, P. S. Obukhov, L. A. Os'kina and I. M. Ustyuzhina

We additionally screened by the tularin test the naturally immune stratum of the population living permanently in this republic. In all, we tested 1733 people from 7 populated centers (Kyzyl--368 people, Tandinskiy Rayon--442, Piy-Khemskiy Rayon--413, Kungurtug village--510). The epicutaneous allergy test with tularin was positive in 2 cases after 48 h. One of them, a professional hunter 42 years of age, lives in the village of Ust-Elegest, but every year he hunts for muskrats in the vicinity of Kungurtug; the other, a rural co-op salesman, is a resident of Kungurtug and is an amateur muskrat hunter. They had not been inoculated against tularemia. We were unable to establish the existence of prior illness that could be suspected of being tularemia. Nevertheless, we tend to believe that the positive skin test with tularin in these cases resulted from prior disease that had gone unnoticed.

By agreement with the Tuva Republic Veterinary Bacteriological Laboratory, we collected for serological testing for tularemia 240 samples of serum from cattle [large horned cattle] from 4 rayons (Kaa-Khemskiy--107, Kyzylskiy--52, Tandinskiy--52, Ulug-Khemskiy--26). Agglutination test was positive (in low titers) with 4 serum samples from animals in Kaa-Khemskiy Rayon (1:80 in 1 case, 1:40 in 1, 1:20 in 2), 2 samples from animals in Tandinskiy Rayon (1:20 in 1 and 1:10 in the other) and 2 in Ulug-Khemskiy Rayon (1:20 in both).

Thus, the results of studies conducted in 1974 on the territory of Tuva ASSR confirmed the existence of endemic sites of tularemia there.

Our future studies should direct themselves to epidemiological and epizootiological distinctions of tularemia in Tuva ASSR, identification of types of endemic sites, their biocenosis and biocenotic links, epidemiological types of morbidity and necessary preventive measures to assure a good epidemiological situation.

## Conclusion

The results of studies conducted in 1974 confirmed the existence of endemic sites for tularemia on the territory of Tuva ASSR.

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# STUDY OF CATALASE ACTIVITY IN F. TULARENSIS

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[Article by I. V. Rodionova, Institute of Epidemiology and Microbiology imeni Gamaleya, USSR Academy of Medical Sciences, Moscow]

[Text] The existing information about the link between catalase activity and virulence of Francisella tularensis is contradictory: Mizuhara reported that there was a direct correlation between these features [6]. Avi-Dor and Janiv [4] failed to demonstrate any correlation in their study of subcultures differing in virulence. A comparison of Yersinia pestis to various strains of microorganisms referable to 23 genera of aerobes showed that lowest catalase activity was referable to F. tularensis and Pasteurella septica [5]. This is virtually the extent of existing studies of this enzymatic system of F. tularensis, and it prompted us to conduct an extensive study of cultures differing in virulence and referable to three geographic races.

We used highly virulent cultures, of which strains No 503/834 and 503/840, 85 and 61 isolated in the USSR, KF, B-300 and 0-458B isolated in the United States, as well as 5 strains of the Japanese variety--Jama, Kosho, Ichijo, Jasoe and Miura--were referable to the Holarctic race. The group referable to the Nearctic race consisted of Schu, strains 0-284, No 8859, Cole and E-261, while the Central Asian strains were No 122, 55, 117 and 543. The minimum lethal dose of these cultures when given hypodermicallly to white mice constituted 1 bacterial cell according to the standard of the State Institute of Standardization and Control imeni Tarasevich.

In addition, we studied Holarctic vaccine strain No 15/10, which has residual virulence for laboratory animals and immunogenicity, and, lastly, avirulent cultures of Holarctic strains No 503 attenuated, No 15 attenuated and No 21/400, Nearctic No 38, R variants produced by Kudelina [2] using erythromycin from Schu and No 543. All of the avirulent strains were nonpathogenic for white mice when given in a dosage of 1 billion bacterial cells, and they were nonimmunogenic.

Most strains were plated from vials on yolk medium, then a 2-day culture was grown on blood agar of fresh fish hydrolysate, with cystine and glucose. The strains were cultivated for 24 h at 37°. In order to recover the required amount of working suspension, cultures of each strain were reinoculated on the same medium, but without blood, and grown for 1 day at 37°. The only exception was referable to the Central Asian strains, for which addition of blood to the above medium was required. We established in preliminary experiments that presence of blood in the media did not affect catalase activity. Several strains were submitted to preliminary passages in guinea pigs: No 503/834 and Schu once, No 85, 55 and 122 three times, No 503/840 and 543 for 6 successive times.

At least two experiments with three concurrent assays of the enzyme in each were conducted with most strains.

Presence of catalase was recorded by the Jolles iodometric method [3]. We used unpurified extracts of bacteria suspensions as the enzyme, after lysis of cells with Tween-20 in saline. To obtain extracts, the F. tularensis cultures were eluated twice in saline with centrifugation (6000 r/min at 4°). We added an aliquot of 1% Tween-20 to a suspension containing 40 billion cells per mℓ, kept the mixture at room temperature for 2 h and allowed it to stand overnight in the refrigerator at 4°. On the following day, we assayed protein according to Lowry in the mildly opalescent supernatant obtained after separation of cells by means of centrifugation at 6000 r/min. When necessary, the extract was diluted to a concentration equaling about 500 μg protein/mℓ.

Specific activity of the enzyme was expressed in micromoles $H_2O_2$ dissociated in 1 min under optimum conditions (pH 6.8), with saturating concentration of substrate (0.005 M $H_2O_2$) in 0.006 M phosphate buffer and temperature of 0°, scaled to 1 mg protein. On the basis of assaying $H_2O_2$ concentration, we calculated the initial rate of the reaction $V_0$ [1] at different intervals (1-3 min) using a graphic method. The results are submitted in units corresponding to 1 μM $H_2O_2$/mg protein/min. Presence of insignificant amounts of Tween-20 in the reaction medium did not affect demonstration of the enzyme.

In analyzing the data obtained, it should be noted, first of all, that all of the F. tularensis cultures examined contained catalase and that the $V_0$ reaction, calculated in parallel experiments for each strain, were identical or differed insignificantly (see Table).

In the experiments, we noted a correlation between catalase activity and the geographic race to which the strains belonged. Maximum $V_0$ were demonstrated in virulent Nearctic strains for which this parameter was in the range of 44.2-84.6 units of activity. The initial rate of the catalase reaction constituted only 17.1-29.9 in typical Holarctic strains, including the American representatives thereof. It is expedient to single out in a special group the Japanese variety of the Holarctic race, which had activity of 34.3-38.7. With the exception of strain No 543, the virulent Central Asian strains had $V_0$ of 44.0-53.1 units, thus being between the Japanese variety and the Nearctic race.

Consequently, our results were not in contradiction with the conception of more intensive energy metabolism in the Nearctic race and, probably, the Central Asian one, which ensues from analysis of data in the literature.

Initial rate of decomposition of $H_2O_2$ by catalase of different strains of F. tularensis

| Race | Strain No | Year isolated | $V_0$ (activity units/g protein) First experiments | $V_0$ (activity units/g protein) Second experiments |
|---|---|---|---|---|
| Holarctic: typical | 503/840*** | 1949 | 29,3 | 29,9 |
| | 503 attenuated | | 3,4 | 3,1 |
| | 85*** | 1974 | 24,6 | 25,1 |
| | 61* | 1974 | 24,0 | — |
| | KF | 1958 | 26,6 | — |
| | B-300 | 1960 | 17,1 | — |
| | O-458B | 1968 | 23,5 | — |
| | 15/10 vaccinal | 1937 | 11,4 | 8,1 |
| | 15 attenuated | | 7,9 | 9,1 |
| | 21/400 * | 1949 | 14,9 | 9,8 |
| Japanese variety | Jama | 1957 | 34,3 | — |
| | Kosho | 1965 | 36,0 | 40,4 |
| | Ichijo | 1975 | 38,7 | — |
| | Jasoe | 1975 | 38,2 | — |
| | Miura | 1975 | 36,3 | — |
| Central Asian | 117 | 1960 | 44,3 | 44,0 |
| | 543*** | 1965 | 35,9 | 35,3 |
| | 543 attenuated | | 10,5 | 10,8 |
| | 122** | 1973 | 53,1 | — |
| | 55** | 1973 | 44,6 | — |
| Nearctic | Schu * | 1941 | 58,7 | 59,5 |
| | Schu attenuated | | 15,9 | 15,2 |
| | O-284 | 1959 | 54,6 | 50,8 |
| | 8859 | 1959 | 49,1 | 44,2 |
| | Cole | 1972 | 67,0 | — |
| | E-261 | 1972 | 84,6 | — |
| | 38 attenuated | 1920 | 13,2 | 15,7 |

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*Culture after one passage in guinea pig.

**After 3 passages.

***After 6 passages.

It is also significant that there is some correlation between level of catalase activity and virulence of cultures. For example, the initial reaction rate in vaccinal Holarctic strain No 15/10 and avirulent strains of this race did not exceed 14.9, i.e., it was almost one-half the mean value for typical virulent cultures. Nearctic avirulent strain No 38, which had a $V_0$ averaging 14.45 units, differed from virulent strains in that catalase activity was one-third to one-sixth of the values for them.

This correlation was particularly well-demonstrable when we compared virulent strains Schu, No 503/840 and 543 to genetically related avirulent subcultures. Also to consider is the fact that the culture of Schu strain used in the first experiments, with some deficiency in virulence that apparently occurred in the course of frequent subcultures while working out optimum conditions for the next experiments, presented significant increase in catalase activity after a single passage in a guinea pig (59.5 versus 15.2 units in the first experiment).

In assessing the experimental data, however, we cannot fail to mention the significant fluctuations of catalase activity in strains within the race groups, which were probably related to the time that had elapsed from the moment a strain was isolated, duration and conditions of storage thereof, frequency of subculturing on nutrient media. Thus, Nearctic strains Cole and E-261, which were isolated in 1972, had higher $V_0$ (67.0 and 84.6, respectively) than strains No 8859 and 0-284, which were isolated in 1959 and had catalase activity of 46.6 and 52.7 units, respectively. Holarctic standard strain No 503/834 and Central Asian No 543, which had undergone numerous subcultures on media and periodic passages in sensitive animals, initially presented low activity of this enzyme (10.3 and 22.1, respectively), without appreciable change in virulence. We succeeded in raising this catalase level to 29.9 and 35.9 after 6 passages in guinea pigs. But already after 3 successive subcultures from yolk medium to agar of fresh fish hydrolysate, activity of catalase dropped to 27.5 in passage strain No 543.

Thus, we demonstrated appreciable differences in catalase activity of F. tularensis strains, which were related to geographic race and virulence thereof. At the same time, we demonstrated fluctuations of levels of this enzyme in cultures of the same strain, which were related to its age and condition.

## Conclusions

1. Catalase was demonstrated in all F. tularensis strains, regardless of their virulence and geographic race.

2. Lowest catalase activity was found in typical Holarctic strains and highest in Nearctic ones; the Japanese variety of the Holarctic race and the Central Asian race occupied an intermediate place according to this parameter.

3. Attenuation of F. tularensis strains led to drastic reduction of catalase activity.

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# QUANTITATIVE LEVELS OF IMMUNOGLOBULINS (A, G, M) IN THE PRESENCE OF DIFFERENT FORMS OF TYPHOID FEVER

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[Article by V. Ya. Lashin, Ye. A. Markova, L. L. Khundanov and L. V. Pogorel'skaya, Second Moscow Medical Institute imeni Pirogov]

[Text] At the present time, much importance is attributed to serum masses of immunoglobulins (Ig), since quantitative changes therein could affect the course and outcome of disease. For this reason, we felt it would be interesting to demonstrate quantitative changes in Ig among patients with typhoid fever, as well as carriers, in order to gain deeper understanding of some aspects of its pathogenesis. There has been insufficient coverage of this question in the literature.

We submit here the results of assays of total Ig in patients suffering from typhoid fever, those who recovered from it, were immunized and essentially healthy subjects, as well as carriers. We tested serum from 309 people (Table 1).

We used the method of simple radial immunodiffusion in gel with monoreceptor antisera against human globulins (IgA, IgG and IgM), produced by the Institute of Epidemiology and Immunology imeni Gamaleya and titrated by the international standard [5], for quantitative assays of different Ig classes.

Of the 96 cases of typhoid fever, 80 were men and 16 women. A mild course was observed in 10 patients (10.6%), moderately severe in 80 (84%) and severe in 6 (6.4%).

The diagnosis of typhoid fever was confirmed bacteriologically in 46 (48%) patients, serologically in 35 (36.4%), and in 15 cases (15.6%) it was determined on the basis of typical clinical signs and confirmed (in 11 out of 12 cases) by the positive result of the intracutaneous test with eberthin (the test was not performed on 3 patients).

At all stages of illness, the level of all classes was higher, with statistical reliability, in blood serum of patients with typhoid fever than in the control ($P<0.05-0.001$). During the early convalescence period (up to the 10th day of normal temperature), the quantity thereof increased ($P<0.05$), as compared to to the acute period; prior to discharge it decreased, but still remained considerably greater than in healthy subjects ($P<0.05-0.001$).

Table 1. Levels of serum Ig (mg/mℓ) in different groups of tested people

| Group tested | Number tested | Total tests | Immunoglobulin class | | |
|---|---|---|---|---|---|
| | | | IgA | IgG | IgM |
| Typhoid fever cases | 96 | 197 | 4,06±0,13 | 20,40±0,53 | 1,84±0,08 |
| Typhoid fever recurrence | 9 | 21 | 4,67±0,22 | 22,84±1,46 | 1,96±0,19 |
| acute carriers | 9 | 30 | 4,86±0,29 | 24,85±1,48 | 1,34±0,13 |
| chronic carriers | 61 | 123 | 4,68±0,12 | 31,5±1,01 | 1,16±0,03 |
| a) phase of isolation | 40 | 82 | 4,86±0,13 | 33,56±1,25 | 1,17±0,09 |
| b) latency phase | 11 | 21 | 4,06±0,22 | 28,0±2,09 | 1,21±0,06 |
| c) transient | 10 | 20 | 4,69±0,3 | 26,15±1,65 | 1,14±0,07 |
| with history of typhoid fever | 43 | 43 | 2,95±0,13 | 16,84±1,29 | 1,12±0,06 |
| Immunized | 59 | 59 | 2,75±0,09 | 15,67±0.6 | 1,35±0,03 |
| Healthy | 32 | 32 | 2,34±0,07 | 12,06±0,51 | 1,16±0,03 |
| Totals | 309 | 505 | | | |

Before being discharged from the hospital, 72 (75%) patients showed virtually no difference in IgM content, as compared to essentially healthy subjects, whereas it was elevated in 24 (25%) cases, which was most often associated with nonspecific bacteremia (in 16 out of 24). IgG and IgA content remained elevated in most patients with typhoid fever, and the increase was quite significant in 19 (19.8%) of them.

We failed to demonstrate an appreciable difference in levels of serum Ig in typhoid fever cases related to methods of laboratory confirmation and severity of the process. IgA level was somewhat higher only with mild course of the disease, as compared to severe and moderately severe.

IgA and IgG of individuals who subsequently became acute carriers were high at all stages of disease, until they were discharged from the hospital. In these cases, we demonstrated more often positive results with the intracutaneous test with Vi typhin, which is inherent in chronic carriers.

Since high levels of IgA and IgG were inherent in acute carriers, which also persisted prior to discharge from the hospital, we deemed it necessary to analyze the case histories of patients who presented higher IgA and IgG levels (19 out of 96) at the stage of late convalescence. Clinical analysis revealed that these patients did not differ in amounts of all Ig classes from acute carriers throughout the illness.

Thus, as shown by the results of our studies, one can detect, already at the acute stage of the disease, patients with high IgA and IgG levels, who could subsequently develop into chronic carriers.

With recurrence of typhoid fever (9 people), we demonstrated high levels of all classes of Ig at all stages of illness, but they dropped virtually to normal in the prerecidivation period.

In chronic typhoid carriers (see Table 1), we observed high levels of IgA and IgG, whereas their IgM content did not differ from that of healthy subjects.

These data confirm once more the previously expounded [2] thesis, according to which the carrier state develops against a background of proper immunological processes.

Table 2. Serum Ig content (mg/mℓ) in patients with typhoid fever (according to stages of illness)

| Stage of illness | Number of patients tested | IgA | IgG | IgM |
|---|---|---|---|---|
| Acute | 48 | 3,12±0,11 | 17,47±0,65 | 1,68±0,74 |
| Early convalescence (up to 10th day of normal temperature) | 55 | 4,52±0,16 | 21,23±0,16 | 1,77±0,09 |
| Late convalescence (before discharge from hospital | 60 | 4,42±0,13 | 19,73±0,7 | 1,52±0,9 |
| Healthy subjects | 32 | 2,34±0,07 | 12,05±0,61 | 1,16±0,03 |
| $P$ | | <0,01 | <0,001 | <0,05 |

Note: The data were submitted to statistical processing on a Minsk-22 computer

In our opinion, the data on Ig levels in so called transient carriers (see Table 1) are of special interest, since the existence of transient carriers has been the subject of debate heretofore. Quantitative levels of the Ig group and incidence of positive intracutaneous tests with Vi typhin, titers of O and Vi antibodies demonstrated in the passive hemagglutination reaction, morphological and histochemical changes in bone marrow [6] of subjects in this group differed insignificantly from the same parameters in latent carriers, for which reason the existence of transient carrier state is questioned.

At the active phase, IgG content was somewhat higher than in the latent phase, which is apparently related to isolation in most cases of the typical form of pathogen, which has high antigenic activity. The demonstrated identity of changes in quantitative levels of Ig in chronic carriers at the isolation [production] phase and latent stage is also indicative of the fact that these two processes are closely interrelated and that they are of some interest to determination of some aspects of pathogenesis of the chronic carrier state [2].

The set of tests we performed (intracutaneous test with Vi typhin, passive hemagglutination with separate demonstration of O and Vi antibodies, bacteriological and immunofluorescence tests of bone marrow, quantitative assays of Ig) enabled us to confirm the opinion we had advanced previously, i.e., that the carrier state is a phasic process, which could be present in the form of latent infection for some time [2]. On the basis of these data, it can be concluded that among individuals who have had typhoid fever there should be some in whom typhoid infection is at the latency phase. For this reason, all subjects who had had typhoid fever were divided into two groups, depending on the results of the intracutaneous Vi typhin test. IgA and IgG content was considerably greater in individuals who reacted positively to Vi typhin than in individuals who had had typhoid fever and did not react to Vi typhin, as well as healthy subjects. A comparison of Ig content in individuals who had had typhoid fever and reacted positively to Vi typhin to chronic carriers in the latent phase failed to demonstrate any differences.

Thus, the results of our studies revealed that the high levels of IgG and IgA in both groups of patients are probably attributable to the same factor, intracellular persistence of the pathogen. This was also indicated by fluorescence in the presence of typhoid antigen in our immunofluorescence study of bone marrow, blood, feces and urine, as well as presence of Vi antibodies in blood serum (passive hemagglutination test), which is what gave us grounds to evaluate patients with positive intracutaneous tests as chronic carriers at a latent phase. It can be assumed that after having had typhoid fever, some of these individuals developed physiological equilibrium between the macroorganism and microorganism--latency phase, which changed to a period of positive findings [isolation] in certain conflict situations, which was demonstrated in surveys of typhoid fever sites or testing of autopsy material [1, 3, 4].

The levels of Ig of the classes in question were higher in individuals immunized against tyhpoid and paratyphoid than in healthy subjects ($P<0.05$), but lower than in patients with typhoid fever.

Thus, our studies enabled us to determine that the chronic carrier state is a dynamic process, which is characterized by high IgA and IgG levels, that start to form already in the acute period. The set of laboratory tests enabled us to demonstrate 28% among those who had a history of typhoid fever who presented the latent phase of the carrier state, which confirms the previously voiced opinion that the actual number of carriers is considerably higher than presently recorded by bacteriological examination of people with a history of typhoid fever.

## Conclusions

1. Clinical and laboratory findings in a study of quantitative levels of immunoglobulins (IgA, IgG and IgM) revealed that the chronic typhoid carrier state is characterized by high levels of IgA and IgG antibodies, whereas chronic carriers did not differ from essentially healthy subjects in levels of macroglobulin antibodies.

2. Chronic (phase of isolation and latency), transient carriers and those with history of typhoid fever who reacted positively to Vi typhin did not differ in IgA and IgG content, and it was high in them. Individuals who had had typhoid fever and did not react to Vi typhin did not differ from essentially healthy subjects with regard to levels of antibodies of the classes in question.

3. Demonstration of high levels of IgA and IgG antibodies, positive intracutaneous tests with Vi typhin, Vi antibodies and specific fluorescence with

typhoid antigen in the immunofluorescence analysis of blood, feces and urine of transient carriers raises doubt as to their existence.

4. The serum Ig content of patients with typhoid fever was higher, with statistical reliability, than in healthy and immunized subjects, and it was unrelated to either severity of illness or laboratory method by which it was confirmed.

5. The acute typhoid carrier state is characterized by high levels of IgA, IgG and IgM, and formation thereof occurs against a background of rather marked immunological reactivity in the patients, with overt lack of "shortage" of macroglobulin (IgM) antibodies.

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# EXPERIMENTAL STUDY OF SUSCEPTIBILITY AND SENSITIVITY TO TULAREMIA INFECTION OF THE ARCTIC LEMMING (DICROSTONYX TORQUATUS PALL.)

Moscow ZHURNAL MIKROBIOLOGII, EPIDEMIOLOGII I IMMUNOBIOLOGII in Russian No 7, Jul 76 (manuscript received 25 Nov 75) pp 110-112

[Article by I. P. Algazin and V. F. Marenko, Omsk Institute of Endemic Infections and Omsk Medical Institute imeni Kalinin]

[Text] It is generally recognized that the intensity of distribution of tularemia infection in wild animal populations is determined by the degree of their susceptibility and sensitivity to infection, as well as population size [4]. With regard to tularemia, only 1-2 groups of animals are significant as sources of infection and hold a leading place in the process of circulation of the pathogen in endemic sites [3].

In the Soviet Union, tularemia was first demonstrated in 1972-1973 among tundra rodents,--lemmings: in the Siberian lemming bacteriologically and serologically, and in the Arctic lemming serologically [2]. The obtained data prompted us to pursue an experimental study of susceptibility and sensitivity to infection of one of these two species, the Arctic lemming.

We used one of the strains previously isolated from lemmings, No 3917, for experimental infection of the animals. Studies of the main biological properties, which were pursued in the Tularemia Laboratory of the Institute of Epidemiology and Microbiology imeni Gamaleya, revealed that this strain is referable to the Holarctic race (Francisella tularensis holarctica Ols.) and is highly virulent for laboratory animals: minimum lethal dose for white mice and guinea pigs is 1 bacterial cell.

In the experiment, we used 15 adult Arctic lemmings weighing 58-60 g. We used each dose on three animals. Concurrently, as a contorl, we infected white mice (in the same quantities). The bacterial suspension was injected hypodermically in the right inguinal region in doses of 100, 10, 1, 0.1 and 0.01 bacterial cells (according to the standard of the State Institute of Standardization and Control of Medical Biologicals imeni Tarasevich). Rodents that died in the course of the experiment were dissected, their lymph nodes, viscera (spleen, liver, brain) and blood were cultured on McCoy's coagulated yolk medium, blood agar with fish hydrolysate and, as a control, beef-extract agar.

Regional and peripheral lymph nodes, in relation to injection site, spleen, liver, lungs, kidneys, heart and brain were submitted to microscopic examination, using conventional methods for histological treatment of tissues and the ordinary panoptic stains (hematoxylin-eosin, picrofuchsin, Nissl and others).

Time of animal death as related to infective dose

| Dose (bacterial cells) | Number of animals used in experiment | Number of deaths (on following days) | | | | Survivals |
|---|---|---|---|---|---|---|
| | | 5th | 6th | 7th | 8th | |
| 100 | 3 | 1 | 2 | 0 | 0 | 0 |
| 10 | 3 | 0 | 3 | 0 | 0 | 0 |
| 1 | 3 | 0 | 3 | 0 | 0 | 0 |
| 0.1 | 3 | 0 | 0 | 2 | 0 | 1 |
| 0.01 | 3 | 0 | 0 | 0 | 0 | 3 |

The studies revealed (see Table) that time of rodent death after infection was related to magnitude of infective dose: with doses of 100, 10 and 1 bacterial cells lemming death occurred on the 5th-6th day, with 0.1 bacterial cell only 2 out of 3 animals died. Analogous findings were made on white mice: 0.1 bacterial cell elicited death of 1 out of 3 infected mice. Dissection of dead lemmings revealed a solid infiltrate at the site of injection of the bacterial suspension, in some places with suppurative dissolution of tissues, enlargement and hyperemia of regional lymph nodes, severe hyperemia of vessels in the surrounding cellular tissue, enlargement and consolidation of the spleen and liver. Cultivation of viscera, lymph nodes and blood on nutrient media (McCoy's and blood agar with fish hydrolysate) revealed that the tissues were strongly contaminated with F. tularensis. Analogous changes in internal organs and results of cultures were observed in the control group of animals.

The changes demonstrated in lemming organs by microscopic analysis were virtually the same as lesions demonstrated in control tests on white mice. Regional lymph nodes presented complete disappearance of usual structural pattern, vessels of both the follicular and paracortical, as well as medullary, zones were dilated showing signs of erythrocyte and plasma stasis. Massive necrotic sites involving the capsule were found against the background of extensive infiltration of lymph node tissue and capsules by neutrophyl leukocytes. Analogous necrotic foci were demonstrable in peripheral lymph nodes and the spleen. In the liver, the alterations appeared more diffuse, due to severe and extensive large-droplet fatty dystrophy of hepatocytes with lysis of nuclei. Multiple bacterial embolisms were found in the hepatic sinusoids. The large vessels presented friable walls infiltrated by round-cell elements. The kidneys revealed severe hemodynamic disorders against the background of extensive granulation dystrophy of the epithelium of the convoluted tubules. Productive vasculitis was encountered in the myocardium, mainly of fine vessels, as well as microsites of necrobiosis and necrosis. The changes in the lungs consisted of dyscirculatory disorders with hemorrhages in the alveolar lumen. There was prevalence of vasocirculatory disturbances in the brain.

Absence of granulomatous reaction in organs was a distinctive feature of the morphological changes demonstrated in lemmings and white mice. Such changes are typical of tylaremia infection of white mice, and from the morphological point of view this determines their high susceptibility and sensitivity to F. tularensis [1]. It should also be noted that we were unable to demonstrate differences in morphological manifestations of tularemia in animals infected with different doses of the pathogen at the final stage of the process. Thus, we established that the Arctic lemming is highly susceptible to tularemia, which determines the importance of this species in epizootiology and epidemiology of tularemia in endemic sites of the tundra type.

## Conclusions

A combined experimental study demonstrated that the Arctic lemming is highly susceptible and sensitive to infection by F. tularensis, and that it is referable to the first animal group (according to Olsuf'yev).

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# INFLUENCE OF SITE OF APPLICATION OF BACTERIAL AEROSOL ON PATHOGENESIS AND SYMPTOMATOLOGY OF AEROSOL INFECTION

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[Article by K. G. Gapochko, Military Medical Academy imeni Kirov, Leningrad]

[Text] We previously demonstrated [2] that the dispersed fraction composition of bacterial aerosol is the principal factor determining retention and primary distribution of aspirated particles in respiratory organs. At the same time, it was established that different species of microorganisms have their own inherent most sensitive regions of the respiratory tract, which are the optimum portal of entry for aerosol infection [3, 9].

As a result, in the course of phylogenesis, determination was made for each infectious disease with an aerosol mechanism of infection of the portals of infection that caused development of a specific clinical pattern. Relatively limited segments of respiratory organs, through which the pathogen penetrates into the body, are inherent in some of them, whereas for others the entire respiratory tract could be the portal of entry of infection.

Diphtheria, scarlet fever and ornithosis (psittacosis) are among the few diseases with phylogenetically limited portals of infection.

Without mentioning the wound variants of disease, clinically marked forms of diphtheria (throat, nose, larynx, conjunctiva) and scarlet fever, in the case of aerosol infection, develop only if the pathogens are applied and accumulate in the upper respiratory tract, conjunctiva and trachea, where the principal pathological focus is formed, which determines the symptomatology of disease. Considering the data on retention and primary distribution of bacterial aerosol in respiratory organs, it can be concluded that infection with diphtheria and scarlet fever occurs by relatively large-particle aerosol with particles of more than 5-10 μm, 80-100% of which are retained and localized in the upper respiratory tract and trachea; the trachea is stricken when even larger particles, over 25 μm in size are impacted on it.

Ornithosis (psittacosis), on the contrary, is characterized by portals of infection situated in the deep parts of the lung, and lung tissue is the site of primary localization and accumulation of the pathogen [12]. McGavran [28] made a comprehensive study of the pathogenesis of psittacosis on M. mulatta monkeys, which were infected with highly dispersed (1-1.5μ m) liquid aerosol of the Borg strain. The infective dose constituted 4000-5000 $LD_{50}$ for mice with intracerebral infection and 100 $LD_{50}$ for monkeys.

Pathohistological examination of the lungs revealed that aerosol infection of monkeys was consistently associated with development of microfocal pneumonia, the foci of which were similar and associated with hyperplasia of tracheobronchial nodes. The pathological process in the lungs started with involvement of respiratory bronchioles equipped with rudimentary alveoli, into which highly dispersed aerosol penetrated. In the second postinfection week, the inflammatory process spread concentrically to peripheral alveoli. On the 10th-16th day, the center of the lesion was localized in respiratory bronchioles and adjacent alveoli, filled with exudate consisting of mononuclears and neutrophils with a small amount of fibrin.

Thus, it can be concluded that, unlike diphtheria and scarlet fever, in the case of ornithosis the portals of infection are only the respiratory parts of the lungs, in particular the respiratory bronchioles, into which highly dispersed (1-2 μm) aerosol particles penetrate and 50-60% retained, causing the typical clinical pattern with localization of the process in the lung parenchyma.

In addition to diseases for which the portals of infection are relatively limited, there are numerous nosological forms, for which although there is a most sensitive zone in respiratory organs, the pathogen is capable to invade the body over the entire respiratory tract, thus eliciting a specific disease. This applies to influenza, measles, smallpox, plague, tularemia, anthrax, brucellosis, glanders, melioidosis, Q fever and deep mycosis, for which the aerosol route of infection is the only one, while the localization of portals of infection has an appreciable influence on the initial phase of pathogenesis and course of disease, often determining its clinical form.

As we know, the clinical course of tularemia, as reflected in the generally recognized classification of Rudnev [10], is closely related to mechanism of infection. Later on, it was determined that, when M. mulatta monkeys are infected with higly dispersed aerosol (1-8 μm), the primary pathological process and bacterial reproduction are demonstrable, as in psittacosis, in the region of the respiratory bronchioles, where specific bronchiolitis develops, which spreads to adjacent lung tissue and involves the alveolar spaces causing development of microfocal pneumonia, which is confirmed histologically and roentgenologically. At the same time, when the animal inhaled monodispersed tularemia aerosol with particles ~18 μm in diameter no lung changes were demonstrable, while the portals of infection were the nasopharynx with involvement of regional lymph nodes, from which the pathogen "broke through" into the blood stream causing hematogenic dissemination and animal death [23].

It must be assumed that the oculobubonic form of tularemia can develop when the conjunctiva is impacted by relatively large (≥25 μm) particles of dust aerosol. The angina-bubonic form, though it is related to water and food outbreaks, can also occur by aerosol infection by dust aerosol, with particles over 5-10 μm in size, which are retained in the nasopharynx and, in a sufficient dosage, can infect the tonsils.

The pneumonic variant of the pulmonary form develops as a result of penetration of particles no more than 1-2 μm in size into respiratory bronchioles and alveoli. Since 40-50% of them are retained upon aspiration, they penetrate expressly into these deep parts of the respiratory tract, causing a specific process in the lung parenchyma.

Rather similar findings are made in the case of aerosol-caused plague. As we know, for a long time the question of pathogenesis of the pulmonary form of this disease was the subject of bitter debates. Bazarov [18] succeeded in producing pneumonic plague by means of insertion of infected cotton sponges in the nasopharynx of guinea pigs, which resembled pneumonic plague in man; however, this experiment could not always be reproduced. At the same time, intratracheal infection of guinea pigs [1, 17, 22, 27] and monkeys consistently produced primary pneumonic plague in these animals.

In addition, when a suspension of Y. pestis was packed into the nose, throat and conjunctiva, as well as with infection of animals (mice, guinea pigs, monkeys) by aspiration of liquid aerosols, the degree of dispersion of which was not determined in the experiments, the experimenters obtained rather contradictory results. Primary pneumonic plague developed only in an insignificant part of the animlas, whereas most of them presented the bubonic form of the disease, with involvement of cervical lymph nodes, acute tracheobronchitis and resulting in fatal septicemia; in some animals, septicemia developed without local lesions in the region of the portals of infection [8, 25, 29, 30, 35].

Numerous experimental data, as well as pathoanatomical findings made at autopsy on patients who expired of plague, enabled Kulesha [6, 7] to formulate his theory of pathogenesis, according to which the portals of entry of infection are the tonsils and lymphoid [tonsillar] ring, from where the pathogen penetrates hematogenically into the lungs and elicits secondary pneumonic plague. The experimental work of Druett et al. [21] provided conclusive clarity to the question of pathogenesis of aerosol-caused plague and pneumonic plague [21]. Primary bronchopneumonia, followed by fatal septicemia, developed in guinea pigs after infection with highly dispersed aerosol, with particles on the order of 1 μm in diameter. At the same time, infection of guinea pigs with aerosol consisting of 12 μm particles led to involvement of lymph nodes of the head and neck, which also ended with fatal septicemia, which developed even faster than in the presence of pneumonia. Infection of monkeys (M. rhesus) with liquid aerosol, with 1-12 μm particles, led to development of primary lobular pneumonia.

Thus, it was demonstrated that after aerosol plague infection, the pathogenesis and clinical signs of disease depended, like tularemia, on localization of portals of infection.

An analogous conclusion is also valid for anthrax. After aspiration of a sufficient dose of highly dispersed aerosol (≤2 μm) by monkeys, B. anthracis spores penetrate into the deep parts of the lungs, invading the lymphatic system on the level of the respiratory bronchioles. This route of infection is not associated with development of primary pathological changes in the lung parenchyma and deep respiratory tract which, in this case, are merely intact portals of infection. Nevertheless, under such conditions, against the background of subsequent sepsis, there is development of signs of disease, the distinctive feature of which is an intrathoracic complex: edema of the lungs and mediastinum, hemorrhagic inflammation of hilum, tracheobronchial and mediastinal lymph nodes (mediastinitis), hemorrhagic pleurisy. When monkeys are infected with a sufficient dose of coarsely dispersed (≥12 μm) aerosol, particles of which are retained in the upper respiratory tract, expressly these parts of the respiratory tract become the portals of infection. When relatively small doses are used for infection, the process may start with the lymphogenic phase, which is clinically associated with lymphadenitis of regional nodes and edema of the face and head; however, it ends with typical hematogenic dissemination, with involvement of internal organs, sepsis and intoxication. With massive infection doses that penetrate into any part of the respiratory tract, or if there is attenuation of the patient's immunobiological resistance, there may not be a lymphogenic phase of anthrax infection and bacteremia can develop right away, evolving into sepsis. Finally, if there is some injury in the respiratory tract of an infected person or animal, or else functional deficiency is present, conditions appear for infection of expressly these regions and for development of primary pulmonary [pneumonic] anthrax [13, 14-16, 19-21, 32].

These data offer convincing evidence of the fact that localization of portals of infection and, consequently, the particulate dispersion of an aerosol have a substantial effect on the clinical course of numerous infectious diseases. Although this question has been studied experimentally for a narrow range of forms of infection, clinical observations convince us that this pattern is much more widespread.

Influenza virus, which is pneumo-epitheliotropic, is always localized in respiratory organs after aspiration. It is believed that expressly the respiratory tract serves as the site of viral reproduction, as well as development of diverse local pathological processes specific to influenza. However, it is assumed that, in some cases, the process could move to the lung parenchyma, causing development of pneumonia differing in form, localization and severity. It is stressed that primary influenzal pneumonia can also develop [4]. Should it not be assumed that, by analogy to previously described diseases, infection by highly dispersed droplet aerosol creates optimum conditions for penetration of virus into deep parts of the lungs and development of primary influenzal pneumonia, whereas coarsely particulate dust aerosol is fixed in the upper respiratory tract, eliciting a less severe form of infection.

Analogous considerations are also valid for measles and primary measles pneumonia.

Adenoviral diseases, for which the aerosol route is the main route of infection, occur in three clinical forms: pharyngoconjunctival fever, acute respiratory disease and pneumonia [5]. We cannot rule out the possibility that, in this case too, along with the distinction of pathogenic properties of different strains of adenoviruses and condition of the macroorganism, primary application and site of pathogen invasion play a substantial role in development of a particular form of the disease.

The portals of entry of infection in the case of aerogenic Q fever infection are primarily the respiratory organs, conjunctiva, as well as gastrointestinal tract into which the aerosol can penetrate. The typical distinction of the disease is the absence of primary pathological changes at the site of penetration of the pathogen in the organism. At the present time, it is believed that Burnet's Coxiella [rickettsia] does not have any definite tropism for different organs and tissues; nevertheless, according to the latest observations, patients with pulmonary lesions in the form of interstitial pneumonia still constitute 5-10% of all clinically marked cases [12]. For this reason, it is again valid to assume that coarsely dispersed aerosol is retained in the upper respiratory tract without eliciting pathological changes in the lung parenchyma, whereas highly dispersed aerosol penetrates into respiratory regions causing development of pneumonia. In view of the fact that spontaneous aerosol infection of man with Q fever occurs chiefly by inhalation of coarsely dispersed aerosol dust, we can understand the relative scarcity of pneumonic forms of the disease.

Thus, we have examined a rather representative group of infectious diseases, for which the localization of portals of entry of aerosol infection had a more or less appreciable effect on the clinical course of illness, causing even development of independent clinical forms in a number of cases.

However, there are nosological forms, for which localization of the portals of entry of infection in the region of the respiratory organs does not affect the clinical course of disease, and as a rule there are no pathological changes at the site of invasion. This applies to transmissive diseases reproduced by experimental aerosol infection: Venezuela and North American equine encephalomyelitis, Rocky Mountain fever and yellow fever. The same group includes typhoid fever (experiments on chimpanzees), botulism (poisoning), as well as many diseases which are not transmitted by the aerosol route in nature. In all these cases, the pathological process is characterized by absence of primary changes in the respiratory system; however, it reproduces entirely the specific clinical signs inherent in these infections in the case of natural routes of infection [24, 26, 31, 33, 36-38].

In conclusion, it should be stressed that there have been particularly comprehensive studies to data of the pathogenesis of aerosol infections caused by highly dispersed aerosols. It was established that the region where infectious particles settle has a significant influence on the outcome of host and microorganism interaction. As we know, there are no purely physical mechanisms for removal of settled particles in the region of the respiratory bronchioles, since the ciliate and mucous layer ends in the region of connection of terminal and respiratory bronchioles. At the same time, it is expressly here that microorganisms are subject to phagocytosis by alveolar macrophages. The bronchiolar region has a particular abundance of blood and lymphatic vessels, as well as lymphoid tissue and rudimentary alveoli along the course of the respiratory bronchus. Numerous experimental studies have shown that highly dispersed aerosols are retained in expressly this region, where development of the pathological process begins [34].

The general patterns pertaining to particle and dispersion of bacterial aerosols, portals of infection and clinicopathogenetic distinctions of disease are not applicable to each specific case. Thus, in particular, when there is a very high concentration of finely dispersed aerosol, infection may occur through the upper respiratory tract, rather than the lungs, or else simultaneously through both these parts of the respiratory tract. At the same time, relatively large aerosol particles, particularly in the case of high concentration and prolonged exposure, could penetrate into the deep parts of the lungs and elicit a pathological process there. It is also known that large aerosol particles can disintegrate in respiratory organs, become finer and thus penetrate deep into the respiratory tract. Finally, one must also take into consideration the active migration of aerosol particles, which occurs due to the activity of the ciliate epithelium and phagocytes.

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# EXPERIMENTAL MIXED TULAREMIA AND OMSK HEMORRHAGIC FEVER INFECTION OF WATER VOLES

Moscow ZHURNAL MIKROBIOLOGII, EPIDEMIOLOGII I IMMUNOBIOLOGII in Russian No 8, Aug 76 (manuscript received 1 Sep 75) pp 118-122

[Article by N. B. Dunayev, Omsk Scientific Research Institute of Endemic Infections]

[Text] With each year, the natural resources of West Siberia and the Extreme North are being developed more and more intensively, for which reason there is more contact between the public and natural sites of zoonoses, to which man is susceptible. Tularemia and Omsk hemorrhagic fever (OHF) are among the most widespread diseases among animals in West Siberia. Although these diseases have been studied rather well, many aspects of their endemicity are still unclear. Thus, there is a need for further investigation of the question of mixed tularemia and OHF infections, epizootics of which had been repeatedly recorded in previous years in populations of muskrats and water voles in West Siberia [5]. No doubt, the possibility of infection of the local inhabitants engaged in agricultural work and other groups is important during epizootic periods. Thus, Sizemova [9] observed concurrent OHF and tularemia in 23 people in an endemic site.

Continuing the studies we started previously [2-4, 6], we investigated mixed tularemia and OHF infection in water voles who, according to our data [1] and those of other authors [7, 8, 10, 11], are not only the principal sources of tularemia but carriers of the OHF virus.

We used a total of 70 water voles in experiments and as a control. They were infected with the Balangul strain of OHF virus, subcutaneously in the internal aspect of the left hind thigh (in a dosage of 100 $LD_{50}$/mℓ) and strain No 503 of F. tularensis, also subcutaneously in the right hind thigh (in a dosage of 1 bacterial cell according to the standard of the Institute imeni Tarasevich). In order to track development of the infectious process, presence and nature of viremia, distribution of viral antigen and F. tularensis in organs and tissues, as well as distinctions of pathomorphological changes in organs, we collected material at different postinfection times, and for this purpose animals were sacrificed, dissected, placing lymph nodes and pieces of organs in 10% neutral formalin and Carnoy solution for histochemical examination.*

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*The morphological examination was made by V. F. Marenko, assistant in the Department of Pathological Anatomy, Omsk Medical Institute.

Concurrently, we prepared impression smears for fluorescence microscopy. We used the bacteriological (McCoy medium) and biological (on white mice) methods of demonstrating and isolating F. tularensis. Virological tests were made on mongrel white mice weighing 6-8 g and on a culture of chick embryo fibroblast tissue (CEF).

The first experimental group of water voles was infected with OHF virus and F. tularensis at a 3-day interval in order to track the course of mixed infection at the height of development of both infections, since with this interval one observes a coincidence of time of maximum development of clinical manifestations of both tularemia and OHF.

Table 1.
Results of virological examination of water vole organs

| Interval between giving OHF virus and F. tularensis | Day, after F. tularensis infection, of testing | Virus titer in blood | Demonstration of viral antigen by method of fluorescent antibodies | | | | |
|---|---|---|---|---|---|---|---|
| | | | brain | spleen | liver | kidney | blood |
| 3 days | 4 | $10^{-4}$ | + | + | + | + | + |
| | 6 | $10^{-5}$ | + | + | + | − | − |
| | 7 | $10^{-4}$ | − | − | − | + | + |
| | 11 | $10^{-3}$ | + | + | + | + | − |
| 7 days | 8 | $10^{-2}$ | + | + | − | − | + |
| | 10 | $10^{-3}$ | + | + | + | + | − |
| | 12 | $10^{-5}$ | − | − | + | − | − |
| | 14 | $10^{-4}$ | − | + | − | + | − |
| | 15 | $10^{-2}$ | − | − | + | + | − |

Note: $10^{-2}$-$10^{-5}$--blood dilutions of 1:100-1:100,000

Visible symptoms of disease appeared in the water voles 3 days after administration of F. tularensis (6th day after inoculation of OHF virus), and they consisted of decrease in motor activity and refusal of feed. The animals died 8 days after inoculation of F. tularensis.

The results of the virological examination revealed that the titer of OHF virus in blood constituted $10^{-4}$ 4 days after infection and reached a maximum on the 6th day ($10^{-5}$). We then observed a decline of viremia, and on the 11th observation day viral titer constituted $10^{-3}$ (Table 1). The method of fluorescent antibodies revealed viral antigen on the 4th, 6th, 7th and 11th days in the brain, spleen, liver, kidneys and blood (see Table 1).

Bacteriological investigation of development of the tularemia process enabled us to isolate F. tularensis cultures 3 days after infection from a regional and peripheral lymph node, as well as spleen; after 4 days from the liver and after 8 days from blood. Analogous results were obtained on subsequent days up to the end of the observation period (Table 2). Using the method of fluorescent antibodies, F. tularensis was demonstrated in a regional lymph node 1 day after infection. F. tularensis was demonstrated in all organs examined from the 3d day after infection to the end of the observation period, and in blood starting on the 4th day (see Table 2).

The biological method yielded analogous data: F. tularensis was demonstrable 1 day after infection in regional and peripheral lymph nodes; in addition to lymph nodes, it was also demonstrated in the spleen and liver after 3 days and in blood after 4 days. Similar results were obtained on subsequent days (see Table 2).

Table 2. Results of testing water vole organs for tularemia

| Interval between giving OHF virus and F. tularensis | Day of test after giving F. tularensis | Demonstration of F. tularensis | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | bacteriologial method | | | | | biological method | | | | | fluorescent antibody method | | | | |
| | | regional lymph node | peripheral lymph node | spleen | liver | blood | regional lymph node | peripheral lymph node | spleen | liver | blood | regional lymph node | peripheral lymph node | spleen | liver | blood |
| | 1 | — | — | — | — | — | + | + | — | — | — | + | — | — | — | — |
| | 3 | + | + | + | — | — | + | + | + | + | — | + | + | + | + | — |
| | 4 | + | + | + | + | — | + | + | + | + | + | + | + | + | + | + |
| | 8 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | 1 | — | — | — | — | — | + | + | — | — | — | + | + | — | — | — |
| | 3 | — | — | — | — | — | + | + | + | + | — | + | + | + | + | — |
| | 5 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| | 7 | + | + | + | + | — | + | + | + | + | + | + | + | + | + | [illegible] |
| | 8 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | — |

Macroscopically, 1 day after giving F. tularensis (4th day after inoculation of OHF virus), regional and peripheral lymph nodes on the side of OHF inoculation were enlarged and invested in a hyperemic capsule. Dilatation of blood vessels was observed at the site of injection of F. tularensis. We must also mention that there was some enlargement of the spleen and dilatation of blood vessels of the meninges and intestine. Dissection after 3 days revealed more significant changes in lymph nodes on the side of administration of OHF virus, considerable enlargement of the spleen, necrotic foci in the liver and areas of hepatization in the lungs. The meningeal blood vessels were dilated, while brain tissue appeared plethoric on sections. The macroscopic changes on the 4th day after giving F. tularensis were all of the same type, unlike those demonstrated previously, with progression of signs of hemorrhagic diathesis and appearance of petechial hemorrhages in the intestinal mucosa, meninges, liver and lungs. Analogous changes, which were associated with appearance of numerous necrotic sites, were observed upon dissection on the 8th day after administration of F. tularensis.

Morphological examination of animal organs 1 day after infection with F. tularensis failed to demonstrate any changes that were typical for either infection. After 3-4 days, signs of tularemia infection appeared in the liver and spleen: granuloma, with necrosis in some of the latter. Appreciable hemocirculatory disturbances were found in other organs. Against this background, structural disturbances of the walls of some small vessels, which are typical of OHF, were noted in brain tissue. This was associated with separation of fibers and plasma saturation. The endothelium proliferated and flaked off, edematous areas appeared around the vessels. We were unable to detect diapedesis of erythrocytes or neuronal damage at this time. But, on the 8th day, there was prevalence of signs of tularemia infection in organs, with predominant involvement in the process of lymph nodes, spleen and liver. There was a drastic increase in number of granulomas subject to breakdown. The vascular disorders were characterized by circulatory disturbances in all areas. The lungs presented severe interstitial-hemorrhagic pneumonia. There was prevalence of circulatory disturbances in brain tissue, and signs of wall lesions inherent in OHF were noted only in some of the fine vessels.

The second experimental group of water voles was infected with OHF virus and 7 days later, when viremia reached a maximum, with F. tularensis. Visible signs of disease appeared on the 6th day after giving OHF virus, whereas deaths were observed 7 and 8 days after inoculation of F. tularensis.

Virologically, OHF virus was demonstrable in blood in a titer of $10^{-2}$ 8 days after administration. The titer rose somewhat, to $10^{-3}$, after 10 days. Viremia reached a maximum ($10^{-5}$) on the 12th day. Thereafter, viral titer dropped: $10^{-4}$ on the 14th day and $10^{-2}$ on the 15th (see Table 1).

By the method of fluorescent antibodies, viral antigen was demonstrable starting on the 8th day after administration of OHF virus in all organs examined; there was none in the liver on the 14th postinfection day and in the spleen on the 15th postinfection day (see Table 1).

We tracked development of the tularemia process for 8 days, since all of the water voles died by this time. Bacteriologically, we succeeded in isolating F. tularensis cultures only on the 5th postinfection day, from regional and peripheral lymph nodes, liver, spleen and blood. Analogous findings were made on subsequent observation days (see Table 2).

We succeeded in demonstrating F. tularensis by the method of fluorescent antibodies in regional and peripheral lymph nodes one day after infection, additionally also in the spleen and liver after 3 days and blood after 5 days. On subsequent days, F. tularensis was consistently demonstrable in all examined organs. Data obtained by the method of fluorescent antibodies were identical to the results of studies by the biological method (see Table 2).

Macroscopically, the changes in organs 1 day after infection with F. tularensis (8th day after infection with OHF virus) were identical to changes observed in animals infected with OHF virus alone, which were examined at the same time; hemodynamic disorders and changes in lymph nodes, both regional to the site of administration of OHF virus and peripheral, on the side of inoculation of virus, were the most prominent. There was hyperemia and enlargement of lymph nodes, considerable plethora of internal organs and enlargement thereof. In the intestine we observed numerous petechial hemorrhages. Examination after 3 days revealed the following, in addition to the above-mentioned changes: consolidation of the lungs, which assumed a dark red color, intensification of plethora of meninges and brain matter with appearance of some hemorrhages. These macroscopic changes were also found in animals 5 days after infection with F. tularensis, whereas 7 and 8 days later we additionally found considerable changes in lymph nodes situated on the side of injection of F. tularemia, which consisted of enlargement, hyperemia and necrosis.

Morphologically, 1-2 days after administering F. tularensis the animals presented only circulatory disturbances in all organs, whereas lymph nodes, mainly on the side of administration of OHF virus, presented hyperplastic reactions in the form of diffuse prolifeation of reticuloendothelium with appearance of "sinus catarrh." The lungs demonstrated lymphohistiocytic infiltration of interalveolar septa. After 5 days, severe dystrophic changes in the liver, which consisted of diffuse granular dystrophy of hepatocytes, were added to these changes. No specific tularemia granulomas were demonstrated. However, on the 7th day, tularemia granulomas of typical structure were found in the lymph nodes, liver and particularly the spleen, which underwent necrosis on the 8th day, with fusion into extensive necrotic fields. Other organs showed circulatory disturbances, to which were added in brain tissue lesions to the walls of fine vessels, which are typical of OHF.

## Conclusions

1. Infection of water voles with pathogens of OHF and tularemia at a 3-day interval elicited development of both infections, without appreciable increase in severity of the pathological process.

2. Administration of F. tularensis 7 days after infection with OHF virus aggravated the course of mixed infection which, according to morphological data, is attributable to intensive progression of the tularemia process at the final stage of the observation period.

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# FIFTIETH ANNIVERSARY OF DISCOVERY OF TULAREMIA IN THE USSR

Moscow ZHURNAL MIKROBIOLOGII, EPIDEMIOLOGII I IMMUNOBIOLOGII in Russian No 11, Nov 76 (manuscript received 20 Mar 76) pp 79-84

[Article by N. G. Olsuf'yev, Institute of Epidemiology and Microbiology imeni Gamaleya, USSR Academy of Medical Sciences]

[Text] 1976 marked the 50th anniversary of the discovery of tularemia in the USSR. In view of this, it is justified to list in brief the main results of studying and controlling this infection in our country.

Tularemia was first diagnosed in 1926 near Astrakhan by Suvorov, Vol'ferts and Voronkova, physicians in the plague-control organization. The outbreak involved about 200 cases, and the diagnosis was confirmed by isolation of the pathogen from 3 patients. In the course of the laboratory work, 4 workers contracted the disease and their serum agglutinated the isolated cultures. The correct diagnosis of tularemia was instrumental in general and intensive development of medical science in the young Soviet republic, development of a network of scientific research institutes dealing with microbiology in our country, in particular the Mikrob Plague-Control Institute, which was staffed by highly qualified specialists.

There is every reason to believe that tularemia had been encountered in our country even prior to 1926, but was diagnosed as some other disease. Thus, there is no question that the outbreak of diseases in 1877 near Astrakhan, which struck about 200 people diagnosed as having a mild case of plague was referable to tularemia.

Already in the early years after discovering tularemia, the medical service attracted attention because of the considerable epidemic outbreaks, which struck hundreds and thousands of people. To date, tularemia is known in 14 Union republics (with the exception of Kirghiz SSR). The distribution of endemic tularemia sites has been established from the western frontiers of the USSR (Kola Peninsula, Karelian ASSR, Baltic region, Kaliningrad Oblast, western Belorussia, the Ukraine and Moldavia) east to the Chukotsk Peninsula, Kamchatka, Sakhalin and Maritime Kray. Tularemia sites to the north are distributed in some areas up to the polar circle and even partially beyond (Gremikha in Murmansk Oblast, Pechora River delta, mouth of the Ob River, Taymyr, northern Yakutia), whereas in the south they are encountered in Transcaucasia and some republics of Central Asia.

The numerous outbreaks of the disease among people, duration of illness and slow convalescence, as well as the wide distribution of endemic sites of this infection in our country, made it necessary to pursue a comprehensive study of tularemia and work out measures to control it.

It is opportune to recall on the 50th anniversary of the discovery of tularemia in the USSR the names of those with whom the main stages of investigation of this infection in the USSR are closely linked: Suvorov, Vol'ferts, Zarkhi, Golov, Khatenever, Sinay, Tumanskiy, Tiflov, El'bert, Gayskiy, Stradomskiy, Rudnev, Bilibin, Drobinskiy, Berinskaya, Karpov, Somov, Pokrovskaya, Vereninova, Novikova, Gromashevskiy, Mayskiy, Yelkin, Faybich, Burgasov, Saltykov, Tinker, Drozhevkina, Popov, Shmuter, Yemel'yanova, Dunayeva, Tsvetkova, Savel'yeva, Uglov, Ananova, Meshcheryakova, Rodionova, Dobrokhotov, Kudelina, Pronina, Mashkov, Sil'chenko, Myasnikov, Borodin, Nekipelov, Bozhenko, Petrov, Maksimov, Naumov, Kucheruk, Domaradskiy, Pilipenko, Aykimbayev, Akhundov, Kondrashkin, Ravdonikas, Antsiferov, Brikman and a number of other researchers.

With respect to institutions, in which tularemia was investigated, we must mention, first of all, the All-Union Scientific Research "Mikrob" Plague-Control Institute, Institute of Experimental Medicine, Institute of Epidemiology and Microbiology imeni Gamaleya, USSR Academy of Medical Sciences, Central Institute for Advanced Training of Physicians, the plague-control institutes of Irkutsk, Stavropol, Rostov-na-Donu, Central Asia, as well as Tomsk Scientific Research Institute of Vaccines and Sera, Rostov Institute of Epidemiology, Microbiology and Hygiene and Omsk Institute of Endemic Infections. The network of oblast, kray and republic-level plague-control stations, which were reorganized in 1955 into departments of particularly dangerous infections of sanitary and epidemiological stations, played a major role in the study and control of tularemia.

In the period in question, Soviet researchers accumulated basic information about the etiology, epidemiology, epizootiology, laboratory diagnosis, pathology, symptomatology, treatment, immunology and prevention of this disease. This knowledge, along with many years of experience in the control of tularemia, served as the foundation for succeeding in lowering drastically the incidence of this infection in our country.

With respect to etiology, comprehensive studies were made of morphological tinctorial, cultural, biochemical, antigenic and pathogenic properties of the pathogen of tularemia. For the first time, by means of electron microscopy, structural details of the cell were identified, determination was made of some enzymatic properties, presence of endotoxin and certain other factors of virulence, nutrient requirements, conditions of in vitro and in vivo reproduction, etc. Sensitivity of the pathogen to exogenous factors and disinfectants was determined. A substantial contribution of Soviet researchers to the study of tularemia was classification of its pathogen as an independent genus, Francisella, and establishment of three geographic races--Holarctic, Central Asian and Nearctic (American)--which differ in pathogenicity and other properties. Variants have been distinguished within the races, in particular, strains of the Holarctic race were found to differ in sensitivity to erythromycin and other antibiotics.

Comprehensive and in-depth investigation of endemicity and epidemiology of tularemia is one of the most important achievements in the study of this infection in the USSR. This was aided by the teaching of Academician Pavlovskiy on endemicity of human diseases. Extensive data on the geography of sites endemic to tularemia were accumulated and submitted to generalization. The natural sources of tularemia infection--water rats, common voles, house mice, hares, muskrats, etc.--that are the main ones for the USSR were identified and studied comprehensively. Secondary infection carriers were defined. In all, 82 species of wild vertebrates were found to contract tularemia in the USSR. Studies were pursued of the patterns of the epizootic process among rodents. Experimentally, the infection was modeled in a significant number of wild mammals and separation thereof into three groups was validated on the basis of their susceptibility and sensitivity to infection, which is important to definition of their role in maintaining endemic tularemia sites. Demonstration was effected of the great importance of Ixodes tickes in transmission and prolonged preservation of tularemia infection in nature. It was also proven that bloodsucking Diptera (mosquitoes, horseflies and clegs [or gadflies]) played an important role as mechanical vectors of this infection.

Spontaneous infection with F. tularensis has been established in the USSR for a total of 74 species of bloodsucking arthropods. Determination was made of the important role of water in transmission of infection among amphibian rodent species, as well as of spontaneous infection of hydrobionts--mollusks, caddis flies and other invertebrates (total of 14 species) in aquatic tularemia sites.

A classification of endemic sites of tularemia based on landform was compiled as a result of accumulation of information about these sites, which included seven main types; the distribution of different types was determined, as well as routes of circulation of infection in them, etc. Methods were developed for forecasting fluctuations in roden population size, since they are the main sources of infection, and this is important to planning of preventive measures. Views have been formulated on paleogenesis of F. tularensis.

Comprehensive studies have been pursued of conditions of infection and routes of transmission of infection to man. A correlation was established between mechanism of infection and clinical forms of disease, which made epidemiological analysis much easier. The patterns of the epidemic process were identified, and the correlation in it between biological and social factors was shown. A classification was developed of epidemiological types of disease (outbreaks), which comprises nine main types and several variants. Most types and variants were described for the first time by Soviet authors, and the classification is the only one in the world to be so complete.

A substantial contribution has been made to the study of pathogenesis and symptomatology of tularemia. Experimental studies have been made of the stages of spreading of F. tularensis in the macroorganism, from the site of invasion to penetration of internal organs; determination was made of intensity of reproduction and level of accumulation of the pathogen in different organs and tissues; morphological, immunological and, in part, pathophysiological macroorganism reactions have been defined. A distinction has been made of phases of the infectious process and a pathogenetic description thereof furnished. The localization of primary lesions and subsequent course of illness were shown in relation to portals of entry of infection. The routes and intensity of discharge of the pathogen in the environment were tracked. A comprehensive study was made of symptoms of tularemia in man and the fullest new clinical classification was prepared of this infection, which includes six main forms (according to localization of primary lesions). The latter have been confirmed experimentally on laboratory animals. Wide studies were pursued of therapy methods, and the high efficacy of antibiotics--streptomycin, chlorotetracycline, etc.--was demonstrated.

In the area of laboratory diagnosis of tularemia, methods of demonstrating this infection in man, as well as wild and domestic animals, bloodsucking arthropods, in water, grain, fodder and other objects, were successfully developed. Determination was made of the high sensitivity and reliability of the biological test on laboratory animals (white mice, guinea pigs) for demonstration of pathogen in material under study. Immunological reactions were investigated comprehensively, and their high specificity was demonstrated. The tularin skin test, which was proposed by Soviet researchers, found wide use in combination with serological tests for diagnosing tularemia in man, whereas at the present time it is used extensively (in the epicutaneous variant) as well for determining the intensity of postvaccinal immunity. A method of immunofluorescence was developed for detection of pathogens in tested material or antibodies in serum. Much attention was devoted to the study of the highly sensitive hemagglutination test in serological diagnosis of tularemia. A new and rather effective method of detecting tularemia epizootics was proposed on the basis of this reaction and introduced to practice, involving demonstration of antigen in bird pellets, droppings of predatory mammals, as well as decayed or mummified rodent carcasses.

As a result of investigation of the antigenic structure of F. tularensis, it was established that it has two antigen complexes: membrane or "superficial-somatic" (Vi) and "somatic" (O). A link was established between virulent and immunogenic properties of the pathogen with the membrane complex. The chemical structure of different antigenic substances was studied. On the basis of comprehensive investigation of variability of F. tularensis, methods were developed for attenuating it and obtaining highly immunogenic vaccine strains that are safe to man and provide a high level of protection against any natural route of infection. Development of a live tularemia vaccine was a major achievement of Soviet scientists, and such a vaccine was not developed abroad. Strain No 15 of Gayskiy turned out to be the most valuable, and it has been used in our country to immunize the public for over 30 years. Comprehensive studies were made of the patterns of formation of immunity to tularemia due to immunization or infection. Determination was made of the mechanisms of immunity related to cellular and humoral factors. Methods were successfully developed for mass cultivation of tularemia vaccine on artificial nutrient media, particularly with use of aeration, lyophilization of vaccine, which assures prolonged preservation, and finally an epicutaneous method of administering the vaccine, which made it much easier to immunize people. In the last few years, a highly effective method of administering the vaccine with a jet injector was developed. It was shown possible to combine immunization against tularemia and brucellosis, plague, smallpox and other infections, without attenuating the immunizing effect of each constituent.

The high efficacy of live tularemia vaccine made it possible to use it as the principal means of preventing tularemia. It was learned that immunization of the public rapidly arrests an outbreak of any origin. The duration of postvaccinal immunity made it possible to move in tularemia sites to scheduled inoculation of the public, with reimmunization every 5 years. It was learned that by immunization alone, one could reliably prevent epidemic outbreaks (but not sporadic cases) in sites of intensive tularemia epizootics among rodents, but for this it is necessary that 90% or more of the public exposed to the risk of infection consist of immune individuals, as ascertained by the tularin skin test. By means of mass scale immunization of the public inhabiting in regions that are enzootic for tularemia, as well as urban residents who travel there, it is possible by means of vaccination combined with other measures to achieve a stable decline of incidence of tularemia among people to isolated cases per year within an oblast, kray or republic. Vaccination is supplemented with such methods as eradication of rodents, prevention of their access to sources of water, foodstuffs, etc. Methods have been developed for eradication of the water rat, common vole and other rodents, which are the principal sources of infection, as well as Ixodes ticks, in order to depress endemic tularemia sites. However, the difficulty of these measures limits their use. Eradication of tularemia sites in some places was the result of developing large reservoirs, with flooding of the habitat of the water rat, thorough drainage [filling] of swamps and other reservoirs, overall plowing of land covering large areas, etc.

The incidence of tularemia among humans in the USSR varied considerably in different years. In the prewar period (1936-1940), when there was no mandatory record of this disease, according to the far from complete data there were an average of 6000 cases of tularemia per year (maximum of 11,000 in 1940). During the Great Patriotic War and the first few postwar years, 40,000-65,000 cases on the average were recorded annually (maximum 136,000 in 1945). Mass scale immunization of the public was instituted in several oblasts starting in 1946, and by 1950 immunizations were used in virtually all regions of the USSR enzootic for tularemia. Since that year, there began a drastic decline in incidence of tularemia among people, and in the last decade (1965-1975) it has held at low levels, an average of about 100 cases per year (maximum of 271 in 1967 and minimum of 22 cases in 1970). Only sporadic cases were noted in most administrative territories, and minor outbreaks much less often. A significant decline in morbidity was achieved, in spite of the tularemia epizootics among rodents, which were observed annually in some republics and oblasts. It can be considered that immunization combined with other measures prevents an average of at least 10,000 cases per year in our country.

The brilliant results obtained in the USSR in control of tularemia require reinforcement in the future, by means of thorough immunization and other measures. Tularemia is an endemic infection, and lowering morbidity among people as a result of mainly immunization does not mean that the endemic sites have been suppressed, since they are widely distributed, quite persistent and, in a number of cases, highly active.

We can advance the following main problems of investigation of tularemia for the next few years: 1) further accumulation of data on nosogeography, with more precise delineation of territories with problem sites and determination of properties of pathogen distributed there; definition of epidemiological breakdown [zoning] of territories; 2) distinctions of the epidemic process and preventive measures during periods of low human morbidity; 3) conditions of existence, epizootiological and epidemiological forecasting, and means of eradicating different types of endemic [enzootic] sites; 4) genetic distinctions factors of immunity and patterns of variability of F. tularensis; providing for stability of vaccine strains stored under laboratory conditions; 5) taxonomy, antigenic structure and biochemical properties; 6) further investigation of pathogenesis and mechanisms of immunity; 7) refinement of laboratory diagnostic methods.

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# EXPERIMENTAL STUDY OF PNEUMONIC FORMS OF PLAGUE, TULAREMIA AND PSEUDOTUBERCULOSIS

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[Article by L. V. Samoylova, A. S. Vasenin, S. A. Piontkovsk.y and Ye. A. Plotnikova, All-Union Scientific Research "Mikrob" Plague-Control Institute, Saratov]

[Text] Clinical and epidemiological observations have established the aerosol route of plague and tularemia infection. It was confirmed experimentally that aerosol infection of animals with pathogens of plague, tularemia and pseudotuberculosis is possible [6, 8, 10, 13, 15, 16, 19, 20, 24, 26].

Experimental investigation of the distribution and reproduction of pathogens of plague, tularemia and pseudotuberculosis in the case of infection via the respiratory tract is important to understanding of the pathogenesis of the pulmonary forms of the diseases. There are few works dealing with the dynamics of the infectious process with aerosol infection of animals with the abovementioned pathogens [5, 12, 21]. Moreover, in the case of tularemia infection, no studies had been made in the first few postinfection hours, and there are only data concerning the increase in number of bacteria after 24 h or later. Fukui et al. [21] observed death of bacteria within 6 h after aerosol infection of guinea pigs with the virulent Alexander strain of Y. pestis, which was grown at 26°, but when bacteria grown at 37° and in vivo were given to guinea pigs, no reduction in number of bacteria was demonstrable within the early postinfection hours.

Our objective was to made a bacteriological study of the dynamics of the infectious process and determine the patterns of reproduction in guinea pigs of virulent pathogens of plague, tularemia and pseudotuberculosis after aerosol infection. Quantitative evaluation was made of the degree of infection of the lungs, regional lymph nodes, blood and viscera by pathogens of these infections. We used virulent strains of Y. pestis--No 231, F. tularensis--No 503--and Y. pseudotuberculosis No 1421 (serotype I) to infect animals. The pathogens were cultivated at optimum temperatures for growth on nutrient media: 28° for Y. pestis, 37° for F. tularensis and 22° for Y. pseudotuberculosis.

Prior to the main experiments, we determined $LD_{50}$ for guinea pigs with aerosol infection and, for comparison, subcutaneous infection. $LD_{50}$ for the pathogens of plague, tularemia and pseudotuberculosis constituted 480 (380-603), 38 (30-56) and $7\times10^4$ $(35\times10^3-14\times10^4)$ bacterial cells with aerosol infection. The figures were 8.9 (6.7-11.5) for the pathogen of plague and $13\times10^4$ $(1\times10^5-175\times10^3)$ for the pathogen of pseudotuberculosis in the case of hypodermic infection. We could not determine LD$_{50}$ for F. tularensis in the case of subcutaneous infection, since 1 DCL [clinically lethal?] constitutes only 1 bacterial cell. It is apparent from these data that LD$_{50}$ with aerosol infection was many times higher than for subcutaneous infection, as determined for plague and tularemia. LD$_{50}$ for the pathogen of pseudotuberculosis was almost twice as high with hypodermic infection as aerosol. The obtained data confirm the opinion advanced by Ogarkov and Gapochko [9], who believe that animal susceptibility to different methods of infection varies, and that different nosological forms have their own specific distinctions. For this reason, in the main experiments we used doses calculated only for aerosol infection.

In the bacteriological study of dynamics of size of bacterial populations, we conducted 2-3 tests with each pathogen. Guinea pigs weighing 250-300 g were submitted to aerosol infection in doses of 50 to 90 LD$_{50}$. The size of aerosol particles constituted 0.5 to 5 μm in 91.5%. The animals were dissected after 30 min, 1, 3, 6, 12, 24 h, 2, 3, 4, 5, 6, 7, 8 and 10 days. We suspended pieces of organs under sterile conditions, ground them in a mortar with sterile sand, the suspension in saline (1:5) was titrated and plated on appropriate solid nutrient media, and it was also used for biotests. Y. pestis and pseudotuberculosis were grown on Hottinger's agar with sodium sulfite, F. tularensis on fish-yeast agar with glucose, cysteine and defibrinated rabbit blood. In addition, to determine the quantity of F. tularensis, we used the biological method of "titration" of organ suspension in white mice [10]. After incubation of cultures for 2-3 days, we counted the colonies, with scaling to 1 g organ weight and 1 mℓ blood. According to the results (see Table), a specific pattern was observed with all three infections. In the first few hours after aerosol infection with the pathogens (3, 6 and 12 h), the number of bacteria in the lungs diminished, as compared to the initial level. With regard to pseudotuberculosis, this pattern was also demonstrated in a study of regional lymph nodes. When animals were infected with plague and pseudotuberculosis, the number of bacteria began to exceed the initial level between the 12th and 24th hour. After infection with tularemia, an increase in number of bacteria occurred between the 24th and 48th postinfection hour. Consequently, the number of Y. pestis, F. tularensis and Y. pseudotuberculosis not only failed to increase for 12 h, but even diminished significantly.

Elimination of bacteria could be attributed to the mechanisms of spontaneous immunity. Bacteria are eliminated from the lungs through the blood and lymphatic systems. In part, they are submitted to phagocytosis by blood macrophages and microphages. Phagocytosis of bacteria by alveolar macrophages, which have hydrolytic lysosome enzymes and perform the function of a fine aerosol filter [1, 22, 25], is another factor of natural resistance. Surfactant (surface-active film), which inherently has bactericidal properties, maintains surface tension of pulmonary alveoli and prevents transudation of fluid into the alveolar lumen, is considered to be significant as a factor of natural protection of the lungs [3, 17, 18, 23]. The outcome of the phagocytic reaction depends not only on functional state of phagocytes of blood, alveolar macrophages and pulmonary surfactant, but largely to extent of aggressive properties in the pathogen, its dosage and virulence. The highly virulent pathogens of plague and tularemia, even in low doses, overcome rather rapidly the natural resistance. In the case of a mildly virulent pathogen, there is more marked resistance. Thus, with use of large doses of Y. pseudotuberculosis for infection, there was slower increase in number of bacteria in the lungs after 24 h and slower subsequent accumulation of the pathogen than when the animals were infected with the pathogens of plague and tularemia.

Results of bacteriological examination of guinea pigs submitted to aerosol infection with the pathogens of plague, tularemia and pseudotuberculosis

| Time of testing | | Quantity of bacteria per gram organ tissue (log) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | plague | | | | | tularemia | | | pseudotuberculosis | | | | |
| | | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 4 | 1 | 2 | 3 | 4 | 5 |
| 1/2 | h | | | | | | 3,0 | | | | | | | |
| 1 | | 3,1 | | 2,3 | | | | | | 4,8 | | | | |
| 3 | | 2,7 | | | | | 2,0 | | | 4,6 | | 2,9 | | |
| 6 | | 2,6 | | | | | 2,0 | | | 3,9 | | 2,0 | | |
| 12 | | 2,2 | | | | | 2,0 | 0,3 | 1,0 | 4,3 | | 2,0 | | |
| 24 | | 4,4 | | 3,2 | 3,1 | 2,1 | 2,0 | 0,3 | 1,0 | 5,4 | | 2,8 | | |
| 2 | day | 7,3 | 2,3 | 6,3 | 4,1 | 3,6 | 5,0 | 1,3 | 5,6 | 6,3 | | 4,2 | | |
| 3 | | 11,4 | 3,4 | 6,4 | 5,0 | 4,3 | 6,0 | 2,3 | 6,6 | 6,5 | | 5,07 | | |
| 4 | | 10,8 | 2,0 | 9,2 | 5,0 | 3,6 | 7,3 | 2,3 | 8,87 | 8,2 | 2,0 | 5,8 | 3,1 | 3,04 |
| 5 | | | | | | | 10,3 | 2,3 | 9,6 | 8,1 | 2,1 | 6,9 | 5.6 | 3,5 |
| 6 | | | | | | | | | | 8,5 | 2,9 | 7.7 | 5,5 | 4,7 |
| 7 | | | | | | | | | | 9,04 | 2,3 | 7,4 | 6,3 | 5,3 |
| 8 | | | | | | | | | | 9,5 | 3,2 | 7,4 | 6,3 | 5,7 |
| 10 | | | | | | | | | | 10,2 | 4,2 | 10,1 | 9,04 | 8,04 |

Key: 1) lungs 2) blood 3) regional node 4) spleen 5) liver

An important pathogenetic factor is generalization of the process and septicemia related to it, which occur on the 1st-2d day with the pulmonary form of plague and tularemia, and on the 4th-5th day with the pulmonary form of pseudotuberculosis. As a result, intoxication of the organism develops, which leads to impairment of vital functions and development of endotoxic shock. The toxic factor emerges very early [2, 7, 11], due to the large absorbing surface of the lungs. When septicemia develops rapidly, fewer septic changes occur in the spleen and liver that could be demonstrated by morphological examination. Such changes do not have time to develop. But our quantitative estimation of bacteria in organs enabled us to demonstrate already developed generalization. Animal death was observed on the 3d-5th day due to pneumonic plague and pneumonic tularemia and on the 7th-10th day due to pneumonic pseudotuberculosis. Death occurred in the presence of tens of billions of bacteria per gram lung and tens of millions and thousands of bacteria per gram of other internal organs. At the stage of generalization and septicemia in the case of pneumonic plague and pneumonic tularemia, no more than several thousand bacteria per milliliter accumulated in blood. Consistent demonstration of bacteria in blood in the presence of generalized infection and the quantity of bacteria in blood and organs warranted the belief that there are much fewer bacteria in blood than in organs. Bacteremia is the consequence of accumulation of bacteria in the primary focus (lungs) and other organs (spleen, liver). An enormous bacterial reservoir is created in organs as a result of their exponential accumulation.

It should be noted that utterly different correlations with regard to localization of bacteria in organs exist in the case of infection through the respiratory tract than with transcutaneous infection. In the latter case, bacteria are concentrated mainly in the spleen, liver and lymph nodes, i.e., in organs with a profusion of reticuloendothelial cells [4, 14]. With aerosol infection, the bacteria first accumulate in the lungs, then penetrate into blood and are localized in the spleen, liver, regional lymph nodes and other organs, then again return into blood. More bacteria accumulate in the lungs, sometimes twice as much as in the spleen and liver.

These studies enabled us to demonstrate a consistent decline in number of bacteria in the lungs, as compared to their initial number, for the first 12 h after aerosol infection with plague, tularemia and pseudotuberculosis. The range of accumulation of the pathogens were determined quantitatively in organs and blood at different stages of the infectious process. The demonstrated dynamics of size of the bacterial population could be used in preparing programs of specific and pathogenetic treatment.

## Conclusions

1. A consistent decrease in quantity of bacteria in the lungs was demonstrated for the first 12 experimental hours after aerosol infection of guinea pigs with Y. pestis, F. tularensis and Y. pseudotuberculosis, after which elimination of bacteria stopped and they began to accumulate in lungs and other organs until the animals died.

2. Generalization of the infectious process and related septicemia occurred on the 1st-2d day in the case of pneumonic plague and pneumonic tularemia, on the 4th-5th day in the case of pneumonic pseudotuberculosis.

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# STUDY OF PHAGOCYTIC ACTIVITY FOR F. TULARENSIS IN HIGHLY SENSITIVE ANIMALS SUBMITTED TO MIXED INFECTION

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[Article by T. N. Dunayeva and K. N. Shlygina, Institute of Epidemiology and Microbiology imeni Gamaleya, USSR Academy of Medical Sciences, Moscow]

[Text] It is known that deviations from typical development of disease are observed in the case of mixed infections. We established that in animal species with little sensitivity to tularemia preinfection with other pathogens activated factors of protection and caused faster development of specific immunity reactions. In such cases, the animals withstood infection with massive lethal doses of F. tularensis.

The intensity of septicemia diminishes and duration of diseases increases in species that are highly sensitive to tularemia after being subjected to mixed infection [5, 6]. The mechanism of these deviations in the course of tularemia have not been identified.

Phagocytosis is one of the important factors of protection of organisms. We studied ingestive and digestive function of blood neutrophils in common voles and white mice infected with Y. pseudotuberculosis and guinea pigs infected with Salmonella. We used the opsonophagocytic reaction (OPR) with homologous bacteria and F. tularensis by a previously described method [7]. We ran the reaction simultaneously on animals with different duration of infection and control (intact) specimens. We examined 4-5 animals in each group. Blood was taken from the heart of voles and mice after sacrificing them and in vivo from guinea pigs.

On the day of running the OPR, animals with different duration of primary infection, as well as control animals, were infected subcutaneously, homolaterally with 0.3 mℓ dilution of F. tularensis culture (strain No 503) containing 10 bacterial cells per milliliter, which corresponded to 1 Dclm [median clinically lethal dose?].

Digestive and ingestive neutrophil function increased not only with regard to homologous bacteria, but F. tularensis in voles infected with Y. pseudotuberculosis in a dosage of 100,000 bacterial cells (Table 1). This intensification was very distinct on the 4th-10th postinfection and less noticeable on the 14th day of pseudotuberculosis infection. Functional integrity of leukocytes persisted for the duration of the experiment in the reaction with Y. pseudotuberculosis, which was related to formation of specific immunity in the infected voles. In intact voles, the index of ingestion of F. tularensis showed a chronographic 2.66-3.54-fold increase, and in the reaction with Y. pseudotuberculosis a 1.33-1.55-fold increase, which reflected the difference in sensitivity of voles to infection with these pathogens.

Table 1. Phagocytic activity of blood neutrophils of voles infected with pseudotuberculosis

| Postinfection day | OPR with F. tularensis | | | | | OPR with Y. pseudotuberculosis | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | ingestion index | | index ratio, 60 min:30 min | phagocytic neutrophils,% | | ingest. index | | index ratio, 60 min:30 min | phagocytic neutrophils,% | |
| | 30 min | 60 min | | 30 min | 60 min | 30 min | 60 min | | 30 min | 60 min |
| 4th | 11,5 | 5,7 | 0,49 | 46 | 23 | 10 | 4,2 | 0,42 | 40 | 17 |
| 7th | 12.2 | 9,2 | 0,75 | 49 | 37 | 10 | 6,0 | 0,60 | 40 | 24 |
| 10th | 12,7 | 6,0 | 0,48 | 51 | 24 | 8,7 | 7,2 | 0.82 | 35 | 29 |
| 14th | 9,2 | 16,5 | 1,78 | 37 | 66 | 6,2 | 4,2 | 0 65 | 25 | 17 |
| Intact: control for 4th, 10th and 14th experimental days | 4,2 | 14,3 | 3,54 | 17 | 57 | 4,5 | 6,0 | 1,33 | 18 | 24 |
| control for 7th day of experiment | 6,0 | 16,0 | 2,66 | 24 | 64 | 7,7 | 12,0 | 1,55 | 31 | 48 |

OPR was done on white mice on the 7th and 11th days after infection with the pathogen of pseudotuberculosis in a dosage of 10,000 bacterial cells. The ingestion index for F. tularensis after 30 min constituted 15.5 and 20.5, and in intact mice 13.2. The reaction indexes rose after 60 min by 1.07-1.5 times in mice infected with Y. pseudotuberculosis and by 2.17 times in intact ones. In the reaction of Y. pseudotuberculosis, the index ratio constituted 1.37 on the 7th day of development of infection and 0.59 on the 11th day. In intact mice, the ingestion index increased by 1.98 times.

All 78 voles infected with F. tularensis on the 4th, 7th, 10th and 14th day after administration of the pathogen of pseudotuberculosis died, and most at the same time as control animals--on the 6th-7th day; 13 voles died on the 8th-10th day. However, in the case of mixed infection, there was a decrease in intensity of invasion of organs and particularly blood, and this was distinctly manifested when the interval between infection was 4-7 days (Table 2). In 11 voles, the results of microscopy of organ smears were negative, and presence of F. tularensis was established only by cultivation on blood agar of Yemel'yanova with penicillin.

Maximum invasion, scored at 3-4 points [9], which is typical for species that are highly sensitive to tularemia, was demonstrated in the spleen of 70.5%

of the voles, in the liver in 52.2% and in blood in only 39.7%. In all cases, invasion of organs and blood of 20 control voles was scored at 3-4. With mixed infection, organ smears showed vacuolized monocytes and in blood neutrophils with phagocytized F. tularensis. Phagocytic cells were found in 26% of the 78 voles that died, in the liver of 36.3% and blood of 32.4%. With intervals of 4, 7, 10 and 14 days between infections, phagocytic cells were demonstrated in 63.6, 57.6, 38.8 and 36.7%, respectively, of the voles that died. In the presence of tularemia infection alone, phagocytic cells were encountered very rarely, due to total lysis thereof and the fact that F. tularensis filled the entire field of vision.

Table 2. Intensity of invasion by F. tularensis of organs of dead voles submitted to mixed infection

| Days between infections, | Number of voles | Demonstration of F.tularensis in smears | | | | | |
|---|---|---|---|---|---|---|---|
| | | spleen | | liver | | blood | |
| | | + | − | + | − | + | − |
| 4 | 22 | 17 | 5 | 12 | 10 | 5 | 17 |
| 7 | 19 | 15 | 4 | 13 | 6 | 7 | 12 |
| 10 | 18 | 16 | 2 | 15 | 3 | 13 | 5 |
| 14 | 19 | 19 | — | 17 | 2 | 15 | 4 |
| Totals | 78 | | 11(14,1%) | | 21(26.7%) | | 38 (48.7%) |
| Control | 20 | 20 | — | 20 | — | 20 | — |

There were 74 deaths out of the 80 mice infected with F. tularensis 3, 5, 7 and 14 days after giving them Y. pseudotuberculosis: 38 died at the same times as control animals, on the 6th-7th day, 22 on the 8th-9th day and 14 mice on the 10th-12th day. There was very intensive invasion by F. tularensis, and bacteremia was scored at 1-2 in only 7 mice. Phagocytosis of F. tularensis was observed in smears of spleen in 6.8% of the 74 animals that died, in the liver in 17.7% and blood in 19.1%. When the intervals between infections constituted 3, 5, 7 and 14 days, phagocytosis was observed in 35.3, 40.0, 27.7 and 1.3% of the mice, respectively. The surviving 6 mice were infected after 20 days with a minimum dose of F. tularensis, and they died on the 7th-10th day.

The decline of septicemia and the incidence of phagocytosis coincided with the period of increase in nonspecific digestive activity of leukocytes during the first 7 days of development of pseudotuberculosis infection. Later on, phagocytosis, which acquired specificity for Y. pseudotuberculosis, had no appreciable effect on reproduction of F. tularensis in vivo and in the animals.

As can be seen in Table 3, we tracked an increase in phagocytic activity in against F. tularensis for a long time in guinea pigs infected with S. typhimurium in a dosage of 500 million bacterial cells: even 54 days after infection, we still observed some bacteriostatic effect of phagocytosis, as compared to intact animals.

Table 3. Phagocytic activity of neutrophils of guinea pigs infected with S. typhimurium

| Postinfection day | OPR with F. tularensis | | | | | OPR with Salmonella | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | ingestion index | | index ratio, 60 min: 30 min | phagocytic neutrophils, % | | ingest. index | | Ind. ratio 60 min: 30 min | phagocytic neutrophils, % | |
| | 30 min | 60 min | | 30 min | 60 min | 30 min | 60 min | | 30 min | 60 min |
| 3 | 12,0 | 8,5 | 0,72 | 49 | 34 | 29,0 | 27,0 | 0,94 | 80 | 80 |
| 7 | 15,8 | 5,7 | 0,35 | 63 | 23 | 29,5 | 24,0 | 0,80 | 74 | 58 |
| 9 | 17,0 | 13,5 | 0,96 | 68 | 54 | 39 | 35,2 | 0,80 | 90 | 95 |
| 14 | 12,0 | 7,7 | 0,66 | 48 | 31 | 38,8 | 32,6 | 0,84 | 90 | 90 |
| 22 | 12,6 | 5.5 | 0,43 | 34 | 26 | 24,4 | 22,2 | 0,95 | 71 | 70 |
| 33 | 10,5 | 18.5 | 1,76 | 42 | 60 | 48 | 31,5 | 0,60 | 98 | 80 |
| 54 | 7,3 | 12,3 | 1,67 | 30 | 48 | 48,6 | 47,0 | 0,90 | 100 | 100 |
| Intact | 6,5 | 16,2 | 2,67 | 25 | 65 | 10,6 | 21,8 | 2,38 | 42 | 76 |

A total of 24 guinea pigs were infected with F. tularensis 3, 7, 9, 14 and 22 days after S. typhimurium. In 12 of them we used the OPR on the 5th day after infection with the pathogen of tularemia. Intensification of digestive activity of neutrophils was found in 8 guinea pigs: the ratio between ingestions indexes after 60 and 30 min of the reaction ranged from 0.47 to 0.93. In 4 guinea pigs, the decrease in bacteria contained in neutrophils was mild: after 60 min the ingestion index remained unchanged or increased by 1.6 times. In control guinea pigs infected only with F. tularensis, the ingestion index showed a 1.8-2.4-fold chronographic increase.

Table 4.
Comparative time of death of guinea pigs demonstrating digestive activity of neutrophils

| Day of death | Numb of guinea pigs | Ingestion index ratio, 60 min: 30 min | | |
|---|---|---|---|---|
| | | >1 | 1 | <1 |
| 4—11 | 22 | 21 | 1 | 0 |
| 12—16 | 26 | 8 | 4 | 14 |
| 17—25 | 15 | 2 | 1 | 12 |

Nine control guinea pigs died of tularemia on the 8th-13th day, on the average on the 11.6th day. Of the 24 guinea pigs submitted to mixed infection, 19 died: 7 on the 10th-13th day, 4 on the 14th-15th day and 8 guinea pigs died on the 17th-25th day. Mean time of death ranged from the 13.6th to 16.3d day in different groups. Blood serum of some of the animals that died on the 17th-25th day showed antibodies to F. tularensis in titers of 1:10, 1:40 and 1:160. No antibodies were found in guinea pigs that died on the 13th-14th day.

No doubt, the protracted course of tularemia after mixed infection was related to intensification of phagocytosis. This can be clearly seen by comparing time of death of the 63 guinea pigs infected with F. tularensis at different intervals after salmonellosis and listeriosis, as well as controls, to the OPR parameters obtained on the day of tularemia infection (Table 4).

The data about surviving guinea pigs are of independent interest. In previous experiments, cases were observed where guinea pigs survived tularemia combined with other infections--salmonellosis, pseudotuberculosis and listeriosis. They demonstrated antibodies to F. tularemia and protracted (up to 40-118 days) F. tularensis carrier state [6].

The five guinea pigs that survived in this experiment were examined on the 32d day after administration of F. tularensis, on the 35th-46th day after infection with Salmonella. It was determined that all of the animals had had salmonellosis: positive agglutination test with serum dilution of 1:160-1:2560. When the agglutination test was done with tularemia diagnosticum and separate examination was made of lymph nodes, spleen, liver and lungs by means of biotests on white mice, no tularemia was demonstrable. We ran the OPR on three guinea pigs prior to infection, and it showed intensive digestion of F. tularensis. The ingestion index constituted 16, 19 and 12 with 30-min exposure and it dropped to 7, 8 and 8 after 60 min. The ratio between indexes constituted 0.43, 0.42 and 0.66. The number of bacteria ingested by 25 neutrophils constituted 86, 115 and 51 in the first smears and dropped to 21, 28 and 31 in the second ones. Intensification of digestive capacity of leukocytes could have caused destruction of a small number of inoculated F. tularensis at the adaptation phase, which is what prevented development of infection. Such instances had been occasionally observed in prior experiments with mixed infection [5, 6]. There were considerably more frequent descriptions of cases of survival of guinea pigs and white mice infected with Y. pestis combined with other pathogens [1, 2, 4, 8, 10]. Bacteriological and serological examination of surviving specimens failed to demonstrate evidence of plague. Probably, in these cases too, "cross phagocytosis" [3, 8] had a devastating effect on Y. pestis during the period of its adaptation in the animal. Not infrequently, such animals are considered "insusceptible," "resistant" or insensitive to infection. In reality, in such cases there is a negligible elevation of threshold of susceptibility due to temporary activation of the nonspecific factor of phagocytotic protection. Reinfection of these animals elicited development of fatal infection.

## Conclusions

1. Infection of animals that are highly sensitive to tularemia with sublethal doses of pathogens of pseudotuberculosis and salmonellosis elicits an increase in ingestive and digestive activity of leukocytes with regard to homologous bacteria and F. tularensis.

2. Activation of the nonspecific protection factor, phagocytosis, causes deviations from the typical course of tularemia, prolonging the disease, reducing intensity of septicemia and, occasionally, raising the susceptibility threshold, which is related to death of small doses of the pathogen at the adaptation phase.

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# A CASE OF TULAREMIA IN AMUR OBLAST

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[Article by N. M. Busoyedova, O. A. Antip'yeva, A. I. Gulyayev, P. T. Moroz and V. F. Dzyubak, Khabarovsk Plague-Control Station, Department of Particularly Dangerous Infections of the Amur Oblast Sanitary and Epidemiological Station]

[Text] Endemic tularemia sites have not been found in Amur Oblast, although individuals who reacted positively in the allergic (average of 0.3-0.4%) and serological (1.4%) tests were encountered among the indigenous population [1]. In 1974, specific antibodies to F. tularensis were demonstrated in a titer of 1:80 in the passive hemagglutination reaction (PHAR) for the first time in field mice and 1:1280 in brown [or Norway] rats which were caught in Arkharinskiy Rayon. These data, as well as the closeness to Amur Oblast of the demonstrated endemic sites of tularemia served as grounds for assuming that they existed there. This assumption was confirmed when a case of tularemia was recorded in September 1975 in the village of Dzhalinda in Skovorodinskiy Rayon.

We submit an excerpt from the case history.

> Patient A., 21 years old, became sick on the morning of 3 September 1975. He experienced headache, general weakness and detected a tender swelling in the left inguinal region. He went to the hospital and was admitted on 17 September.
>
> Upon admission, 2 lymph nodes, 3×2 and 1.5×1.5 cm in size, were palpable in the left inguinal region, they were not tender, of solid elastic consistency and not adherent to the skin. Similar lymph nodes (the size of a bean) were found on the left side of the neck over the posterior surface of the nutatory [sternocleidomastoid?] muscle and on the right, under the scapula. No traces of portals of entry of infection were found. Upon admission and during the patient's 2-month stay in the hospital his temperature was normal. There were no complaints of weakness, perspiration, poor appetite or impaired sleep. The disease was in a mild form.

> Blood tests on 18 Sep and several times later (24 Sep, 8 Oct and 12 Nov) failed to reveal deviations from normal.
>
> Between the time he was admitted and 23 Oct, the patient received only multiple vitamins and ascorbic acid. After the diagnosis of tularemia was established, antibiotics were ordered: tetracycline in a dosage of 200,000 AU 4 times a day from 24 to 31 Oct, and streptomycin in a dosage of 500,000 AU [active units] twice a day from 31 Oct to 10 Nov. While the patient was hospitalized, there was a reduction in size of lymph nodes on the neck and under the scapula, and they were barely palpable at the time of discharge (19 Nov).

At first, the multiple enlarged lymph nodes caused suspicion of a blood disease; for this reason, the enlarged inguinal nodes were completely removed on 2 October, and one of them was submitted to histological examination, while the other was sent for bacteriological testing.

Macroscopic examination revealed the following: the nodes consisted of bean-like elements 2.5×2 cm in size, of flexible consistency, watery and yellowish on section. Microscopic examination revealed that the capsule of the lymph node was fibrous, structural pattern was intact, fine and rather large foci were found mainly under the capsule, some of them in the form of nodules consisting of epithelioid and clear reticular cells. Tiny necrotic foci filled with disintegrating leukocytes were visible in the center of many nodules. In one section, an element was found in the nodular region that resembled a giant multinuclear cell.

The origin of the above changes in the lymph node was not quite clear; they resembled pseudotubercular nodules and in some places presented elements of tuberculous eruptions, so that it was necessary to bacteriologically rule out pseudotuberculosis, as well as to give antituberculosis medication.

After this diagnosis was made, the Khabarovsk Plague-Control Station was informed about the patient and on 23 October (49th day of illness) blood was taken for serological testing for tularemia and pseudotuberculosis; the cutaneous tularemia test was made and one of the resected lymph nodes was taken for bacteriological examination. The allergy test was positive (hyperemia, infiltrate 1.0×1.0 cm in size) after 24 h. Blood serum revealed antibodies to F. tularensis: titer of 1:160 in the agglutination reaction (AR) and 1:2560 in the PHAR. When samples were taken again on the 69th and 97th days, antibody titer was 1:1280 in the PHAR, 1:160 and 1:40, respectively in the AR. No specific antibodies to the pathogen of pseudotuberculosis were demonstrated.

Suspension of lymph node tissue was used for hypodermic infection of 4 white mice, which died on the 9th-10th day. Dissection revealed hyperemia of vessels of subcutaneous cellular tissue, enlargement and hyperemia of regional (inguinal) lymph nodes; 2 mice presented enlargement and consolidation of the spleen and the other 2 failed to demonstrate an visible changes in the spleen.

A culture was isolated from the animals that died, which was typical in tinctorial and cultural-morphological properties for F. tularensis: the pathogen did not grow on the usual nutrient media, it was agglutinated by antitularemia agglutinating serum (from the Irkutsk Plague-Control Institute) in a dilution of 1:800 (serum titer 1:3200), did not contain citrulline ureidase and did not ferment glycerin. The minimum lethal dose of culture for white mice and guinea pigs constituted 1 bacterial cell with subcutaneous infection. The species of the strain was confirmed by the Tularemia Laboratory of Irkutsk Plague-Control Institute.

Thus, on the basis of the positive tularin test, presence in the patient's blood serum of specific antibodies to F. tularensis and isolation of the pathogen from a lymph node, the diagnosis of tularemia was made on patient A. This case is unquestionably local, since the patient had not traveled anywhere for 1 year. During the last 10 days of August he often walked barefoot, in a T-shirt and shorts, and had noticed numerous mosquito bites.

Results of retrospective screening of the inhabitants of Dzhalinda for tularemia

| Group examined | Number of people tested | Positive results of | | | |
|---|---|---|---|---|---|
| | | tularin test | | PHAR | |
| | | absolute | % | absolute | % |
| Timber management workers | 123 | 8 | 6.4 | 8 | 8 |
| School children | 303 | 1 | 0.3 | 1 | - |
| Totals | 829* | 71 | 8.5 | 64 | 61 |

*Translator's note: Numbers are copied as they were in source, but they do not seem to jibe.

Among the tested inhabitants of Dzhalinda, 8.5% showed a positive tularin test and their blood serum presented specific hemagglutinating antibodies in titers of 1:20 to 1:320 (see Table).

The results of this screening warrant the assumption that the subjects with positive reactions were infected somewhere in the vicinity of Dzhalinda. This is indicated by the fact that of the 8 timber management workers who had positive reactions 4 were indigenous inhabitants and the others had lived in the village for 10 15 years. It should be noted that positive reactions were demonstrated in a large percentage of the groups that were the most exposed to the risk of infection because of their occupation (see Table). The history of subjects with positive test results showed no mention of prior illness that clinically resembled tularemia.

Thus, in the vicinity of the village of Dzhalinda, near the national frontier, there is an endemic tularemia site, which can apparently be classified as the eastern, floodplain-swamp type, since this locality consists to a significant extent of the swampy region along the banks of the Amur River.

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# DYNAMICS OF IMMUNOGLOBULIN M AND G SYNTHESIS IN THE PRESENCE OF EXPERIMENTAL TULAREMIA

Moscow ZHURNAL MIKROBIOLOGII, EPIDEMIOLOGII I IMMUNOBIOLOGII in Russian No 7, Jul 77 (manuscript received 27 Sep 76) pp 123-126

[Article by I. S. Meshcheryakova, Institute of Epidemiology and Microbiology imeni Gamaleya, USSR Academy of Medical Sciences, Moscow]

[Text] The established heterogeneity of antibodies and possibility of differentiating them according to immunoglobulin classes make it possible to assess more completely and deeply the immunological changes that occur with various infections or vaccinal processes, determine involvement of IgM and IgG in serological reactions, as well as to determine the predominant link between different classes of immunoglobulins and specific diseases.

At the present time, the patterns of synthesis of different classes of immunoglobulins have been studied in relation to many viral and bacterial infections. These studies made it possible, in particular, to demonstrate that the presence and levels of IgG in serum could serve as an indicator of activity and severity of an infectious process, permitting more accurate determination of the etiological factor of disease and differentiate with sufficient reliability between postinfection and postvaccinal antibodies [1-3, 5-8].

Such differentiation of antibodies and identification of the role of different classes of immunoglobulins to the immunological process are quite important in tularemia infection. The serological and allergic diagnostic methods that exist at the present time for this disease do not permit detection of differences in immunological reactions of individuals who sustained infection and those who were immunized, which makes it difficult to assess the epidemiological situation in endemic tularemia sites and detect previously unknown sites of infection.

We conducted a study on rabbits weighing 2.5-3.0 kg in order to make a comparative evaluation of the nature and dynamics of antibodies synthesized in the presence of infectious and vaccinal processes referable to tularemia. The animals were infected with virulent F. tularensis strains No 55 (Francisella tularensis mediaasiatica) and 503/840 (F. tularensis holarctica), while vaccine strain 15/10 NIIEG [Scientific Research Institute of Epidemiology and Hygiene] was used for immunization (5 animals per group). The virulent strains were injected subcutaneously in a dosage of 10 million bacterial cells and the vaccine subcutaneously in a dosage of 1 billion bacterial cells. Dynamic tests were made on the animals' serum: after 7, 14, 21, 30 days and then at 1-month intervals for up to 6 and 11 months in different groups of animals. In all we tested more than 100 samples of serum by two serological methods: agglutination reaction (AR) with bacterial diagnosticum and passive hemagglutination reaction (PHAR) with erythrocytic diagnosticum.

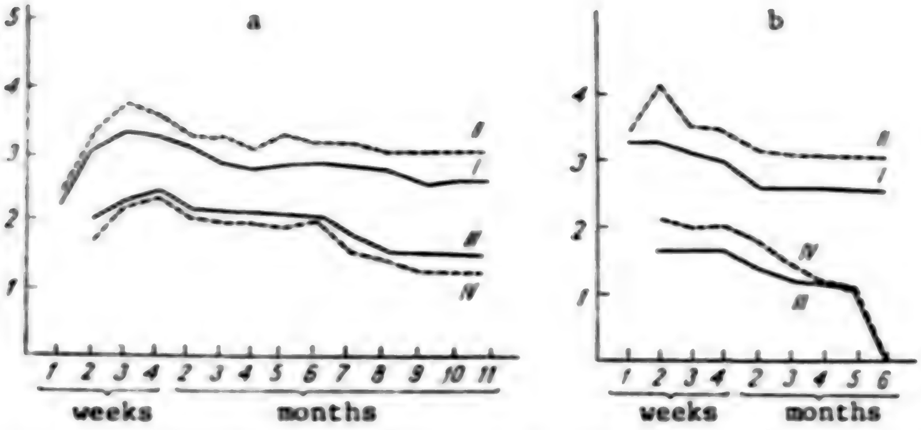
We used the simplest and most accessible method of treating antibody sulfhydryl bonds with reducing agents in order to differentiate between IgM and IgG. This led to selective breakdown of macroglobulins without having an appreciable effect on activity of microglobulin antibodies. We used cysteine hydrochloride (by the method of Chernokhvostova [4]) and 2-mercaptoethanol (by the method of Deutsch and Morton [9]) as reducing agents. Before treatment, we assayed overall or total antibody titer and after treatment, the IgG titer. We determined the presence of IgM on the basis of total absence of immune antibodies in the treated specimens of 4-fold or greater decline of titers in the sera, as compared to the control.

We failed to demonstrate any basic difference in indicators of the immunological reactions between treatment with the two reducing agents; however, it is preferable to use 2-mercaptoethanol which, unlike cysteine, does not form a sediment that makes it difficult to evaluate results. We submit below the results we obtained with use of 2-mercaptoethanol.

In animals infected with virulent strain No 55, only IgM was demonstrable 1 week later in average titers of 1:240 and 1:300 in the AR and PHAR, respectively. At this time IgG was not yet demonstrable; 2 weeks after infection they were synthesized and consistently demonstrable in infected animals, mean titers constituting 1:104 in the AR and 1:72 in the PHAR. We also found an appreciable elevation of overall antibody level in serum, which reached a maximum 3 weeks after infection: 1:2936 with the AR and 1:7040 with the PHAR. Highest IgG titer was recorded after 1 month, averaging 1:384 (AR) and 1:288 (PHAR). Subsequent long-term observation revealed gradual decline of overall antibody titers, which constituted 1:640-1:1280 after 11 months (duration of observation). It should be noted that throughout the study, IgG were demonstrable in blood; their titers also gradually decreased constituting 1:40-1:60 after 11 months, there being no appreciable difference between the AR and PHAR; in a number of cases the titer was lower in the PHAR than AR (see Figure).

Analogous studies of antibody structure and dynamics in rabbits infected with virulent strain 503/840 revealed the same basic patterns, some differences being noted only in mean antibody titers.

Thus, at the early stage of the infectious process after tularemia infection there is intensive production of IgM. IgG appear against the background of maximum macroglobulin synthesis (2d-3d week), reach a maximum by the 4th week and are demonstrable in the animals' blood for a long time. It was found that the PHAR is more effective for demonstration of IgM and AR for IgG. Since IgM are the original type of antibodies and first to appear in response to antigenic stimulation, expressly the PHAR is recommended for early detection of tularemia.



Dynamics of antibodies (mean titers) in infected (a) and immunized (b) rabbits; x-axis--testing time; y-axis, mean titer log

I, II) AR parameters before and after treatment of serum with 2-mercaptoethanol

III, IV) PHAR parameters

We demonstrated appreciable differences in nature and dynamics of antibodies when we examined immunized animals. IgM were consistently demonstrable in rabbit serum 1 week after immunization in both serological reactions, with mean antibody titers of 1:2280 (AR) and 1:3804 (PHAR), which reached a maximum after 2-3 weeks--1:2680 (AR) and 1:12,160 (PHAR)--after which the concentration gradually diminished, constituting 1:120 (AR) and 1:1600 (PHAR) by the 6th month. As in infected animals, the immunized ones showed IgG only after 2 weeks; however, the titers thereof remained low throughout the observation period (they did not exceed 1:40-1:160 in both reactions), declined rapidly and were not demonstrable after 6 months. In a number of cases, no IgG were demonstrable in immunized animals over the entire observation period.

Thus, we succeeded in establishing differences in structure and levels of immunoglobulins, as well as in time of their circulation in blood of infected and immunized animals. IgG production is attributable main[illegible] the infectious process. This warrants the assumption that IgG are an indi[illegible] intensity of the immunological response in the presence of tularemia. [illegible] vaccinal process elicited chiefly production of IgM; IgG were demonstrable inconsistently, in low titers and disappeared faster from blood of experimental animals.

The different proportions of IgM and IgG in serum of infected and immunized animals can apparently serve as a criterion for differentiation between infectious and vaccinal processes by serological tests, and they permit more accurate determination of the immunological status of the organism in the presence of tularemia.

Conclusions

1. Appreciable differences were found in IgG level and time of persistence thereof in blood of animals infected with F. tularensis and immunized against tularemia.

2. The passive hemagglutination reaction was found to be more effective for studying IgM, whereas clearer results were obtained with the agglutination reaction for determining IgG.

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# CYTOPATHIC EFFECT OF FRANCISELLA TULARENSIS ON CULTURE OF PERITONEAL MACROPHAGES

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[Article by T. N. Maslova and R. A. Savel'yeva, Institute of Epidemiology and Microbiology imeni Gamaleya, USSR Academy of Medical Sciences]

[Text] It has been demonstrated that virulent Brucella strains, as well as Shigella flexneri, are capable of eliciting death of macrophages cultivated in vitro [1, 3, 7], and for dysentery bacteria this capacity is related to the presence of cytotoxin [3]. Studies with a culture of macrophages and F. tularensis revealed differences between the behavior of virulent and avirulent cultures. These data are of interest in connection with investigation of the virulence of F. tularensis [8-12]; however, the information in the literature on this matter is contradictory.

[page 105 missing]

... At different postinfection times (3 to 48 h), sample test tubes were removed from the incubator, pH of the medium, cloudiness were recorded and preparations made. The latter were fixed in methyl alcohol and stained according to Romanovsky-Giemsa. We observed simultaneously washed and unwashed preparations, both experimental and control ones.

In the first series of experiments we determined the behavior of the abovementioned strains of F. tularensis in a culture of peritoneal macrophages from intact guinea pigs. The experiments enabled us to determine that the degree of ingestion by macrophages of all F. tularensis strains was relatively the same. After incubation for 5 h, there were marked qualitative and quantitative changes in the cell population of macrophage cultures infected with vaccinal and virulent strains. However, morphological cell changes were more marked. Regardless of the quantity of F. tularensis present in the cytoplasm of macrophages, the latter were damaged so severely that there was no question of irreversibility of the dystrophic changes.

F. tularensis ingested by macrophages elicited primary degeneration of karyoplasm with subsequent breakdown of cytoplasmic structures of cells. The most typical and frequent form of dystrophy was karyorrhexis and vacuolization of nuclear substance (Figure 1, a, b [photo not reproduced]). The cytotoxic effect of bacteria on cells was unrelated to intensive intracellular reproduction of the pathogen. A small amount of bacterial cells ingested by a phagocyte, some of which were at the stage of digestion, was sufficient for manifestation of the cytotoxic effect of F. tularensis on macrophages. This was indicated by the eluated preparations, which made it possible to rule out repeated phagocytosis. By 24 h, there was complete degeneration of cellular elements. In their place we usually encountered accumulations of bacteria, the shape of which repeated the outline of the former cell (Figure 2 [photo not reproduced]). The fibroblastoid or stromal elements, which appeared in the macrophage culture at this time, were quite resistant to F. tularensis: completely destroyed macrophages and completely intact fibroblastoid cells could be seen (Figure 3 [photo not reproduced]).

Having become convinced that all of the strains had a markedly destructive effect on macrophages, we took a culture of strain No 503 killed by heat. When macrophages were infected with killed bacteria there was active phagocytosis, followed by complete digestion of ingested bacteria, without death or damage to macrophages.

The results of these experiments were indicative of very great lability of macrophages from animals sensitive to F. tularensis. The enzymatic systems of phagocytes were inadequate, even in relation to the vaccine strain, which had residual virulence and an antigen complex similar to that of virulent strains.

Negative results were obtained from experiments conducted to demonstrate the effect of bacterial metabolic products on macrophages through the nutrient medium: the cellular elements of macrophage culture did not undergo any visible morphological changes, and they were in the same condition as control elements.

A second series of experiments was conducted on albino rat macrophages for a comparative study of the toxic effect of F. tularensis on cells from an animal that is not very sensitive to this infection. It was shown that cells from rats did not undergo the marked pathological changes inherent in cultures of guinea pig macrophages. The total number of cells virtually failed to decline after cultivation for 24-48 h. We observed intensive phagocytosis of all strains: after 24 h there were signs of active digestion of captured bacteria, and the macrophages presented no appreciable signs of degeneration. We were impressed by the condition of the nuclear system, which presented no visible changes in this case (Figure 4 [photo not reproduced]). Thus, cells taken from insensitive animals were not subject to appreciable cytopathic effects, even of virulent strains of F. tularensis.

The results of our studies warrant the assumption that F. tularensis has a specific factor responsible for its cytopathic effect on macrophages, and this is consistent with previously obtained data concerning the existence of a toxic component in F. tularensis. The nature of this factor and mechanism of resistance of macrophages from insensitive animals are presently being studied.

## Conclusions

1. It was shown that F. tularensis strains differing in virulence had a marked cytotoxic effect on macrophages from animals sensitive to this infection.

2. Macrophages from animals who are not sensitive to tularemia were more resistant to F. tularensis.

3. A culture of F. tularemia killed by heat was submitted to active phagocytosis by macrophages, without causing death of the latter.

4. The cytotoxic effect of F. tularemia is attributable to a factor in the nature of a toxin.

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# MECHANISM OF CHANGES IN REACTIVITY OF ANIMALS TO TULAREMIA AFTER MIXED INFECTION

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[Article by T. N. Dunayeva, Ye. V. Ananova and K. N. Shlygina, Institute of Epidemiology and Microbiology imeni Gamaleya, USSR Academy of Medical Sciences, Moscow]

[Text] Preinfection of animals with sublethal doses of certain pathogens has an inhibitory effect on development of tularemia [1]. The mechanism of this effect has not been determined.

## Material and Methods

We made a comparative study of the pathogenesis and immunogenesis of listeriosis and salmonellosis in albino rats, as well as the effect of these infections on rat sensitivity to tularemia. In the experiments, we used Listeria monocytogenes strain No 681 serotype 1/2a, Salmonella enteritidis No 165 and standard Francisella tularensis strain No 503. Animals were infected subcutaneously with a sublethal dose of Listeria or Salmonella (500 million bacterial cells). We made a dynamic study of distribution of the pathogen in organs, intensity of plasma reaction in a regional lymph node and accumulation of specific antibodies in blood serum. We used cortisone-treated white mice for isolation of Listeria.

## Results and Discussion

We demonstrated differences in the course of listeriosis and salmonellosis in rats, and in intensity of immunological reactions. Listeriosis had a brief course. Already on the 10th-15th day, the rats were free of the pathogen, and only a few specimens presented regional lymph nodes, from which Listeria were isolated. Salmonella was demonstrable in the spleen and liver up to the 10th day and in some cases up to the 18th experimental day. Salmonella persisted in lymph nodes of some rats for 38-58 days.

Appreciable differences were observed in intensity of plasma cell production. In rats with listeriosis, the increase in number of plasmoblasts did not lead to significant increase in number of plasmocytes. Maximum quantity thereof, which did not exceed 30-54 cells per 50 fields of vision, was recorded on the 6th-8th day. By the 10th experimental day, there was a decrease in quantity of plasmocytes (Table 1). Salmonella infection elicited intensive development of the plasmocyte reaction, and immunomorphological changes lasted for a longer time. Maximum quantity of plasmocytes was noted on the 4th-10th day, reaching 112-360 cells per 50 fields. On the 28th day, the quantity of plasmoblasts and plasmocytes was greater in rats with salmonellosis than those with listeriosis.

Table 1. Dynamics of plasmocyte reaction and accumulation of antibodies in blood serum of albino rats infected with Listeria and Salmonella (dosage 500 million bacterial cells)

| Post-infection day | Listeriosis |  |  | Salmonellosis |  |  |
|---|---|---|---|---|---|---|
|  | plasmoblasts* | plasmocytes* | agglutin. react.** | plasmoblast* | plasmocyte* | agglutin. react.** |
| 1 | 11 | 2 | —*** | — | — | — |
| 2 | 95 | 15 | — | 74 | 20 | — |
| 3 | 131 | 15 | 12,6 | — | — | — |
| 4 | 97 | 20 | 25,2 | 135 | 53 | 80 |
| 7 | 71 | 24 | 40 | 97 | 157 | 640 |
| 10 | 38 | 13 | 0—40 | 86 | 65 | 845 |
| 15—20 | 37 | 10 | 0—40 | 38 | 23 | 124 |
| 28 | 29 | 10 | 0 | 38 | 24 | 0—160 |
| Intact | 38 | 2,7 |  |  |  |  |

*Arithmetic mean per 50 fields of vision.

**Geometric mean (reciprocals of antibody titers).

***No tests done (-).

Accumulation of circulating antibodies was consistent with differences in the immunomorphological reaction: brief and minimal with listeriosis, prolonged and sizable with salmonellosis. Already on the 4th experimental day, reaction titers reached 1:320 in some animals, and maximum titers were 1:2560 on the 7th-10th day. Antibodies in serum dilution of 1:40-1:320 were demonstrated in some rats 58 days after infection.

Concurrently with examination of some parameters of pathogenesis and immunogenesis of listeriosis and salmonelosis, we determined rat sensitivity to tularemia. The rats were infected with lethal doses of F. tularensis homolaterally. Concurrently, control animals were infected with the same doses. The inhibitory effect of listeriosis on development of tularemia infection was brief, and it was manifested when the interval between infections was 2 to 10 days (Table 2).

We observed heightened sensitivity to tularemia in rats with salmonellosis even when both were inoculated simultaneously, and it persisted for 1 month (Table 3). It should be stressed that the survival rate in experiments where intervals were 2-10 days was the same when tularemia was combined with listeriosis and salmonellosis. With a dosage of 100 million bacteria, 69.3% of the rats with listeriosis survived and 60.5% of those with salmonellosis. After infection with 1 billion F. tularensis, 16.2 and 20.5%, respectively, survived. When the interval between infections was 18 or more days, the death rate among rats with listeriosis was the same as in the control, whereas among those with salmonellosis 63 and 20% of those infected with 100 million and

1 billion F. tularensis survived. Consequently, the main difference was in duration of heterologous immunity due to development of the background infections.

Table 2. Inhibitory effect of listeriosis on tularemia in albino rats

| Interval between infections, days | F. tularensis infective dose, quantity of bacterial cells | Infected (numerator) and deaths (denominator) | Time of death, day | Surviving rats | Control: infected (numer.) & deaths (denom.) | Control: time of death, day |
|---|---|---|---|---|---|---|
| 0 | 100 million | 6/4 | 2–3 | 2 | 6/4 | 2–3 |
|  | 1 billion | 6/6 | 2–3 | 0 | 6/6 | 2 |
| 2 | 100 million | 10/1 | 19 | 9 | 13/13 | 2–3 |
|  | 1 billion | 10/10 | 2–9 | 0 | 13/13 | 2–3 |
| 4 | 100 million | 25/6 | 2 | 19 | 10/10 | 2–3 |
|  | 1 billion | 23/17 | 2–3 | 6 | 10/10 | 2–3 |
| 7 | 100 million | 6/3 | 4–7 | 3 | 6/4 | 2–3 |
|  | 1 billion | 6/6 | 2–3 | 0 | 6/6 | 2 |
| 10 | 100 million | 6/3 | 3–11 | 3 | * |  |
|  | 1 billion | 6/5 | 2–3 | 1 |  |  |
| 18 | 100 million | 5/4 | 2–3 | 1 | 6/4 | 2–3 |
|  | 1 billion | 5/5 | 2 | 0 | 6/6 | 2 |
| 32 | 100 million | 5/4 | 2–3 | 1 | * |  |
|  | 1 billion | 5/5 | 2 | 0 |  |  |
| Totals: | 100 million | 59/21 (35,6%) |  | 38 (64,4%) | 41/37 (90,2%) |  |
|  | 1 billion | 59/52 (88,2%) |  | 7 (11,8%) | 41/41 (100%) |  |

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*See control for preceding time.

We can relate the prolonged change in reactivity of rats infected with Salmonella to the longer activation of their cellular reaction. It is known that the presence of antigen during development of immunocompetent cells is significant to determination of immunological specificity of these cells [3]. Administration of new antigen at intermediate stages of histogenesis could cause rapid appearance of immunocompetent cells that produce antibodies to this antigen--in our experiments tularemia antigen.

This phenomenon is well-demonstrable when rats are infected with a moderate dose of Salmonella--1000 bacterial cells--then F. tularensis. In this experiment, the immunomorphological reactions of rats developed more slowly than after infection with 500 million bacteria. On the 4th day, the mean quantity of plasmoblasts in a regional lymph node increased to only 34 per 50 fields, while plasmocytes remained at a low level, only 2 cells. Accordingly, there were no antibodies to Salmonella antigen in blood serum. On the 18th day, the number of plasmoblasts increased to 43 and plasmocytes to 27.5. Antibody titer was in the range of 1:10 to 1:320 (geometric mean 1:56.7). In this experiment, when rats were infected with lethal doses of F. tularensis they presented the same increase in resistance to tularemia as rats infected with a massive dose of Salmonella. The surviving rats were examined on the 20th day after tularemia infection. No antibodies to Salmonella were demonstrable in rats infected at a

4-day interval, in spite of the presence of bacteria in lymph nodes, spleen and, in one case, the liver. Titers of antibodies to F. tularensis ranged from 1:80 to 1:640.

Table 3. Inhibitory effect of salmonellosis on tularemia in albino rats

| Interval between infections, days | F. tularensis infective dose, quantity of bacterial cells | Infected (numerator) and deaths (denominator) | Time of death, day | Surviving rats | Control: infected (numerat.) and deaths (denomin.) | Control: Time of death |
|---|---|---|---|---|---|---|
| 0 | 100 million | 10/4 | 3 | 6 | 10/10 | 3 |
| | 1 billion | 5/4 | 3 | 1 | 5/5 | 3–4 |
| 2 | 100 million | 21/6 | 2–5 | 15 | 10/10 | 3–4 |
| | 1 billion | 10/4 | 3–11 | 6 | 5/5 | 2–4 |
| 4 | 100 million | 10/2 | 3–9 | 8 | * | |
| | 1 billion | 5/4 | 2–3 | 1 | | |
| 7 | 100 million | 20/11 | 3 | 9 | 10/10 | 2–4 |
| | 1 billion | 11/11 | 2–3 | 0 | 5/5 | 2 |
| 10 | 100 million | 10/4 | 3 | 6 | 10/10 | 2–5 |
| | 1 billion | 5/5 | 2–3 | 0 | 5/5 | 2–3 |
| 18 | 100 million | 20/6 | 3–11 | 14 | 10/10 | 3–6 |
| | 1 billion | 10/6 | 2–6 | 4 | 5/5 | 2 |
| 28 | 100 million | 10/5 | 2–3 | 5 | * | |
| | 1 billion | 10/10 | 2–3 | 0 | | |
| 58 | 100 million | 10/10 | 2–3 | 0 | 10/10 | 2–4 |
| | 1 billion | 7/7 | 2–3 | 0 | 5/5 | 2 |
| Totals: | | | | | | |
| | 100 million | 111/48 (43,2%) | | 63 (56,8%) | 60/60 (100%) | |
| | 1 billion | 63/51 (80,9%) | | 12 (19,1%) | 30/30 (100%) | |

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*See control for preceding time.

Antibodies to both pathogens were present in rats infected with tularemia at an interval of 18 days: to F. tularensis in titers of 1:160-1:320 and to Salmonella in titers of 1:10-1:160. Consequently, administration of a massive dose of F. tularensis during the period of proliferation of plasmoblast cells (4th day) caused production of antibodies to the new antigen and inhibited production of antibodies to the first pathogen.

When F. tularensis was given to animals who already developed an immunological reaction to salmonellosis, we observed the opposite phenomenon: stimulation of production of antibodies to the first antigen. In rats infected with a massive dose and examined on the 22d day (20th day after tularemia infection) titers of antibodies to Salmonella reached 1:1280 (geometric mean 1:440). In the case of monoinfection, maximum antibody titer was 1:640, while the geometric mean constituted 1:124 (see Table 1). On the 31st day after administration of Salmonella (13th day after tularemia infection), antibody titers ranged from 1:40 to 1:1280 (mean 1:276), whereas in the case of monoinfection antibodies were not demonstrable in all of the rats and their titers did not exceed 1:160.

Reactivation of production of antibodies to listeriosis antigen was even more graphic. After a massive dose of Listeria, all 12 surviving rats, which were tested on the 20th day after tularemia infection (24th, 27th and 30th days after Listeria infection), presented antibodies to Listeria in serum dilutions of 1:40-1:1280 (geometric mean 1:300). Antibody production was much higher than after Listeria infection (see Table 1). Intensification of anamnestic serological reactions to heterogeneous pathogens has been described in great gerbils infected with plague [2] and rabbits immunized with Staphylococcus [4]. Activation of development of immunocompetent cells makes it possible to express within a short time the genetically inherent capacity of white rats to produce antibodies to F. tularensis. As we know, presence of antibodies enhances phagocytosis, which is important in the system of protective forces of the organism.

## Conclusions

1. Experiments on alibno rats infected with Listeria or Salmonella and then F. tularensis demonstrated differences in duration of nonspecific resistance to tularemia, which are attributable to the distinctions of pathogenesis and immunogenesis of the background infections.

2. Activation of immunomorphological reaction, which causes faster development of specific reactions of immunity to tularemia, is one of the important factors that enhances rat resistance to this infection after being submitted to mixed infection.

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# EFFICACY OF TULAREMIA ANTIBODY ERYTHROCYTIC DIAGNOSTICUM FOR DEMONSTRATION OF SPECIFIC ANTIGEN AND ANTIBODIES

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[Article by M. F. Shmuter, M. A. Aykimbayev, A. M. Yelyubayeva, D. I. Brikman and Ye. F. Charnaya, Central Asian Scientific Research Plague-Control Institute, Alma-Ata]

[Text] A method was developed for commercial production of tularemia antibody erythrocytic diagnosticum in liquid and dry form at the Central Asian Scientific Research Plague-Control Institute in 1969-1972; TU-42 [technical specifications] and laboratory instructions for production of this product were compiled [1]. The results of experimental investigation were indicative of high sensitivity and specificity of this product for demonstration of both antigen in the passive hemagglutination reaction (PHAR) and antibodies in the antigen neutralization reaction (ANR) [1-3].

Following a test program coordinated with the State Institute of Standardization and Control of Medical Biologicals imeni Tarasevich, which was approved by the Committee for Vaccines and Sera of the USSR Ministry of Health, this diagnosticum was tested at the Irkutsk Scientific Research Plague-Control Institute of Siberia and the Far East and at the Omsk Scientific Research Institute of Endemic Infections. The results of this testing are submitted here.

Experimental series of the product, in dry and liquid form, were prepared for the trials. Specific Vi antigen content was assayed with this diagnosticum in 97 cultures of F. tularensis killed by boiling, as well as 114 cultures of other microorganisms (Y. pestis--40, Listeria--12, Pasteurella--12, Erysipelothrix--8, Brucella--17, Salmonella and E. coli--25). In addition, 313 suspensions of liver, spleen and extracts of femoral bones from 209 white mice that died of tularemia and 104 control mice were tested for specific antigen. For these tests, we used fresh, decayed and dry animal carcasses.

In order to check the expediency of using tularemia antibody erythrocytic diagnosticum for demonstration of specific antibodies with the ANR, we analyzed serum from 92 people who had been immunized or infected, rabbits and guinea pigs, as well as 102 control sera (from human donors--40, rabbits--20, guinea pigs--24, brucella -- 12, cholera--4 and tularemia agglutinating--2).

All of the material was coded by a special commission and analyzed in coded form.

The results of testing 211 cultures in the PHAR for presence of specific antigen confirmed the high sensitivity and strict specificity of tularemia antibody erythrocytic diagnosticum. PHAR with the tested diagnosticum was positive with suspensions of 97 tularemia cultures. The suspensions of the other bacteria listed above did not react with this diagnosticum.

Table 1. Sensitivity of F. tularensis strains in PHAR

| Diagnosticum | Number of cultures | Results of PHAR with different concentr. of cultures (thous.bacterial cells/0.2 mℓ) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 31 | 50 | 62 | 100 | 125 | 200 | 250 | 500 | geom. mean titer |
| Liquid | 97 | 2 | 1 | 11 | 13 | 18 | 31 | 19 | 2 | 147,6 |
| Dry | 97 | — | 2 | 6 | 4 | 18 | 39 | 25 | 3 | 175,4 |

Table 2. Results of testing suspensions of organs from white mice infected with tularemia

| Dilution of material (thousand-fold) | PHAR with suspensions | | | | Dilution (thous) | PHAR with femur extract (70) | |
|---|---|---|---|---|---|---|---|
| | liver (69) | | spleen (70) | | | diagnosticum | |
| | diagnosticum | | | | | | |
| | liq. | dry | liq. | dry | | liquid | dry |
| 1:8 | — | — | 1 | 2 | 1:0,8 | — | — |
| 1:16 | — | — | 2 | 3 | 1:1,6 | 1 | 2 |
| 1:32 | 1 | — | 5 | 3 | 1:3,2 | 4 | 2 |
| 1:64 | 2 | 3 | 21 | 23 | 1:6,4 | 10 | 11 |
| 1:128 | 11 | 12 | 23 | 22 | 1:12,8 | 15 | 15 |
| 1:256 | 26 | 38 | 17 | 16 | 1:25,6 | 15 | 12 |
| 1:512 | 29 | 16 | 1 | 1 | 1:51,2 | 25 | 28 |
| Titer geometric mean (thousand-fold) | 1:283 | 1:246,8 | 1:103 | 1:97,5 | | 1:19,8 | 1:20,4 |

In the PHAR with the tested product, F. tularensis strains were found to vary in sensitivity (Table 1). Shmuter et al. [4] had previously advanced a thesis to the effect that sensitivity of diagnosticum is related to virulence of cultures (presence of Vi antigen). In order to confirm this, killed suspensions of 2 tularemia cultures--virulent Schu of the Nearctic variety and the avirulent reference strain 21/400 in concentrations of 1 million and 1 billion bacterial cells per milliliter were studied at the Irkutsk Institute. A positive PHAR was obtained with Schu strain in a dosage of 100,000 cells, whereas with strain 21/400 it was negative even with a dosage of 200 millions cells/0.2 mℓ. This indicated that one could indirectly assess the virulence of F. tularensis according to sensitivity of the diagnosticum.

The results of testing 219 suspensions of organs from tularemia-infected white mice were indicative of the high specificity and sensitivity of tularemia antibody erythrocyte diagnosticum (Table 2). Positive results with the hemagglutination test, with suspensions of spleen and liver of 1:2000 and suspensions of femurs of 1:200, were not obtained with any of the 104 such suspensions from control animals.

The results of examining suspensions of white mouse organs were indicative of a higher antigen concentration in samples from decomposed and dry organs and lower in samples taken from fresh material, from infected dead mice. Such distribution of antigen is apparently related to the fact that there is less antigen per unit weight of fresh material than in dry and decomposed material. We were impressed by the higher concentration of antigen in the liver than the spleen, although usually more bacteria are isolated in bacteriological tests of the spleen than the liver. This distribution of antigen could apparently be attributed to the fact that, concurrently with reproduction of F. tularensis in the liver, there was destruction thereof and accumulation of antigen, not only of F. tularensis that multiplied in the liver, but antigen that had migrated from other parts of the body with blood.

Table 3. Results of testing serum of infected and immunized people and animals (ANR)

| Titer | Serum from infected rabbits (20) |  | Titer | Serum from |  |  |  |
|---|---|---|---|---|---|---|---|
|  |  |  |  | immunized guinea pigs (57) |  | sick and immunized people (12) |  |
|  | diagnosticum |  |  | diagnosticum |  |  |  |
|  | liquid | dry |  | liquid | dry | liq. | dry |
| Negative | — | — | Negative | 6 | 6 | 3 | 3 |
| 1:400 | 1 | 1 | 1:40 | 4 | 6 | 2 | 2 |
| 1:800 | 7 | 6 | 1:80 | 17 | 18 | 3 | 3 |
| 1:1600 | 9 | 10 | 1:160 | 17 | 16 | 2 | 1 |
| 1:3200 | 3 | 3 | 1:320 | 12 | 10 | — | 1 |
| 1:6400 | — | — | 1:640 | 1 | 1 | 2 | 2 |
| Titer geometric mean | 1:1299 | 1:1345 |  | 1:126 | 1:124 | 1:127 | 1:127 |
| Total positive reactions, % | 100,0 | 100,0 |  | 89,5 | 89,5 | 75,0 | 75,0 |

These results were indicative of the strict specificity and high sensitivity of tularemia antibody erythrocytic diagnosticum and its suitability for demonstration of specific Vi antigen, in both cultures of F. tularensis and suspensions of organs from rodents that died of tularemia, regardless of condition of the carcasses. There was virtually no difference between liquid and dry diagnosticum with respect to sensitivity.

We also explored the possibility of using the diagnosticum in the ANR in order to demonstrate specific antibodies in blood serum from healthy, sick and immunized people and animals. Antibodies in a titer of 1:64,000 were found in tularemic agglutinating serum, in reactions with both dry and liquid diagnosticum. Specific antibodies were also demonstrable in serum from infected and immunized people and animals (Table 3). In control serum--Brucella, cholera agglutinating and serum taken from healthy rabbits, there were no specific antibodies. Antibodies in titers of 1:40-1:80 were found in only 2 out of 24 serum specimens from healthy guinea pigs and serum from 3 healthy people. Testing of the serum from these people in the PHAR with tularemia antigen diagnosticum revealed antibodies in the same titers. These data indicated that, in the cases mentioned, the people had had an anamnestic reaction due to prior illness or immunization.

These data were indicative of considerable specificity and relatively high sensitivity of the ANR with tularemia antibody erythrocytic diagnosticum and the possibility of using it in order to detect antibodies in people and animals who had been sick and immunized.

However, considering that it is more difficult to run the three-element reaction (ANR) than the two-element one (PHAR), use of tularemia antibody erythrocytic diagnosticum for demonstration of specific antibodies should be recommended only in the absence of antigenic diagnosticum, as well as for running two mutually checked reactions--PHAR with antigenic diagnosticum and ANR with antibody diagnosticum.

## Conclusions

1. The PHAR with tularemia antibody erythrocytic diagnosticum is a specific and highly sensitive test, and it is suitable for demonstration of specific antigen in both bacterial suspensions and suspensions of organs and tissues from animals that died of tularemia, regardless of condition (fresh, decomposed or dry carcasses).

2. The PHAR with tularemia antibody erythrocytic diagnosticum makes it possible to indirectly assess the virulence of tested cultures of F. tularensis.

3. Tularemia erythrocytic antibody diagnosticum can be used in some cases for demonstration of specific antibodies in serum from people and animals who were previously sick and immunized by means of running the antigen neutralization reaction.

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# ROLE OF LEMMINGS IN ENDEMIC TULAREMIA SITES IN THE ARCTIC REGION

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[Article by I. P. Algazin and I. I. Bogdanov, Scientific Research Institute of Endemic Infections, Omsk]

## [Text] Introduction

Among the endemic mammalian species of the subarctic region that inhabit exclusively the tundra or forest-tundra, there is wide distribution of rodents represented by three vole species of the genus Microtus and four lemming species (3 of the genus Lemmus and 1 of the genus Dicrostonyx). The enormous territory of the Eurasian tundar, from Arkhangelsk to Chukotka is populated primary by two lemming species: Siberian (Lemmus sibiricus Kerr.), which is the most numerous and inhabits the main tundra biotopes, and Arctic (Dicrostonyx torquatus Pall.), of which there are much fewer than the Siberian lemming, inhabiting the relative dry and elevated tundra regions [12].

Observations of Soviet and foreign scientists established that episodes of mass-scale reproduction, recurring every 3-4 years, which are associated with sizable migrations and death of most animals, are inherent in lemmings, and as a result their number occasionally decreases to 1/300-1/400th [10, 14].

When analyzing factors that affect the dynamics of rodent population size, in recent years much importance has been attributed to intrapopulation regulatory mechanisms [1, 4, 15], among which a place is reserved to deaths due to epizootic diseases, including tularemia [5]. It was possible to confirm, for the first time, the tularemic etiology of lemming death during a period of mass reproduction in one of the provinces of Lapland, in Sweden [13]. The author assumed that the cases of tularemia among humans in that locality were due to a tularemia epizootic in the lemming population.

Only indirect data were obtained for the territory of the USSR, which indicated a possibility of tularemia among these rodent species: intensive deaths during years of a "peak" in their number, coincidence of cases of the disease among the inhabitants with periods of mass reproduction and death of the animals, results of retrospective epidemiological analysis [9, 11]. Maksimov [7] proposed that a distinction be made of the tundra type of endemic tularemia site, in the belief that the lemming is the principal carrier of infection in this type of site.

In 1972, one of the present authors participated [2] in a study of lemmings trapped in the western part of Taymyr Peninsula, in the region of the Pyasina River, between the village of Valek and the estuary of Pura River, and in the lower reaches of the Pura, which yielded distinct seropositive data indicative of contact of these animals with F. tularensis. This served as grounds for a 4-year (1973-1976) study at a permanent base of an endemic tularemia site in East Taymyr, in the vicinity of the Novorybnoye trading post, in a typical moss and lichen tundra (73° north latitude). The area of the territory included in the study constituted about 27 $km^2$. The Siberian lemming settlements were in the humid lowlands, whereas settlements of the Arctic lemming were situated on elevations.

## Material and Methods

Over the above-mentioned period, a total of 1013 lemmings were tested for tularemia, including 949 Siberian and 64 Arctic lemmings; in addition we examined 210 bird pellets, 29 mummified rodent carcasses, 126 samples of water from open reservoirs in the tundra and 128 samples of wet substrate collected near rodent nests. Finally, we examined 8300 bloodsucking mosquitoes and 4941 gamasid ticks.

The lemmings were caught using Gero press-type traps (equipped with a trip cord [tripping device]), which were placed in lines of 100, 150 or 200 traps at an average distance of 5 m from one another. Gamasid ticks were collected by brushing lemmings, as well as from nests using "thermoeclectors." We used a biological test on white mice by the conventional method to isolate F. tularensis from rodents, environmental objects and arthropods. Biotests with gamasid ticks taken from animals were made immediately after brushing them out. Ticks of the nest-burrow complex were kept alive before the tests in nests, maintaining an optimum microclimate. Cultures of internal organs and blood of biotested animals that died were made by the impression method on coagulated yolk medium, blood and (for a control) beef-extract agar. Serological testing of pellets and mummified carcases for tularemia antigen was performed using the antibody neutralization reaction (ANR). Specific antigens were demonstrated in rodents by means of the usual agglutination reaction and passive hemagglutination reaction with tularemia erythrocytic diagnosticum.

## Results and Discussion

During the period of our observations, the number of lemmings changed in the following manner: In 1973, there was an average of 29 Siberian lemmings per 100 trap-days and 2 Arctic lemmings per 100 trap-days. In 1974, there was a complete depression of both species: we failed to trap a single animal in the vicinity of the station, nor did we find any fresh traces (droppings, evidence of gnawing, fresh nests and tunnels). In 1975, a new rise in number of animals began. There was an average of 3 Siberian lemmings and 0.6 Arctic ones per 100-trap-days, and the latter were trapped not only on elevations, but humid lowlands, i.e., the typical habitats of the Siberian lemming. The number of animals continued to grow in 1976: average of 9.9 Siberian lemmings and 7.2 Arctic ones per 100 trap-days, and we no longer observed penetration of the Arctic lemming into the habitats of the Siberian one.

Results of analysis of bird pellets and mummified lemming carcasses in North Taymyr

| Year | Where material was collected | Pellets | | | Mummified carcasses | | |
|---|---|---|---|---|---|---|---|
| | | total | positive results | | total tested | positive results | |
| | | | absolute | %±t | | abs. | %±t |
| 1974 | Syndassko | 116 | 61 | 52.56±4.63 | 13 | 4 | 30.76±12.8 |
| 1974 | Novorybnoye | 9 | 2 | 22.22±13.85 | 3 | - | - |
| 1975 | " | 21 | 6 | 28.56±9.85 | - | - | - |
| 1976 | " | 64 | 25 | 39.06±6.09 | 13 | 7 | 53.84±13.9 |

A total of 17 taxons of gamasid and acariform ticks were associated with lemmings and their nests, 4 of which were the most numerous: Laelaps lemmi Urube, Haemogamasus ambulans Thorel, Hirstionyssus isabellinus Oudms, Parasitus netskyi Pav. et Bogd. The first was encountered only on animals, the second and third on both animals and in nests, and the fourth usually in the nests.

During the first increase in number of animals (1973), 6 strains of F. tularensis were isolated, for the first time in the Soviet Union, from Siberian lemmings (5 cultures from live animals and 1 from a carcass). In 1974, when there were no live animals at the station, bacteriological tests were made of water samples from the moist substrate near nests referable to summer settlements of lemmings. In two cases, we succeeded in isolating a strain of F. tularensis. In 1974, during a period of gradual increase in animals, a culture of F. tularensis was isolated from water samples from a swampy region near settlements of Siberian lemmings. In 1976, 2 strains of F. tularemia were isolated from Siberian lemmings (1 from a live animal and 1 from a carcass), and again a culture of the microorganisms was isolated from a water sample from the substrate near a nest.

Examination of biological properties of all of the strains showed that they were referable to the Holarctic race of the pathogen (Francisella tularensis holarctica Ols.).

Data from a serological analysis for tularemia of bird pellets and mummified lemming carcasses (see Table) can serve as a graphic illustration of the dynamics of the epizootic process. In 1974, very few pellets and carcasses were collected in the vicinity of Novorybnoye. At the same time, according to statements of local residents, there was a migration of lemmings in the fall and spring, mainly to the north. Near the Syndassko trading post, which is north of Novorybnoye, on the coast of Khatanga Bay of the Laptev Sea, a large number of mummified carcasses and pellets was collected.* There was a high percentage of pellets and carcasses containing specific antigen, and ANR titers reached 1:1280. This warranted our assumption that there had been an intensive tularemia epizootic among lemmings in East Taymyr, in 1973. According to data in the literature [6, 8], epizootics had also been observed in the same year on Yugorsk Peninsula, Yamala and West Yakutia, i.e., they involved an enormous territory. In 1975, the percentage of pellets with antigen was considerably lower, ANR titers ranged from 1:20 to 1:640, while no cultures were isolated from animals. Considering the small number of lemmings, it is difficult to assume that there had been any significant epizootic at that time. In 1976, the quantity of pellets and carcasses containing antigen increased again, and antigen was demonstrable in suspension dilutions of up to 1:1280; cultures of F. tularensis were isolated from the lemmings. Considering the growth in number of animals, there is reason for us to talk about the start of a significant epizootic, which could be the cause of lemming depression in 1977.

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*V. A. Il'in collected the material near Syndassko.

The pathogen is transmitted via different routes in the lemming population. For example, we think the water factor is important to prolonged preservation of F. tularensis in the environment, which is added by the stable, low-temperature condition of tundra reservoirs and moist substrate near nests. The settlements of Siberian lemmings are in the humid lowlands, for this reason, many of their tunnels, burrows and spaces near nests are sometimes filled with seeping ground water. The feed tables are often near the very edge of small bodies of water. With a large number of rodents, these substrates, which are infected by the excreta of sick animals, could serve as a source of infection. In our opinion, isolation of F. tularensis cultures from water in a year of depression [of lemming population] serves as proof of the significance of water as the medium where the pathogen is preserved during periods of absence of lemmings.

Consumption by the lemmings of other lemmings and carcasses thereof may be another route of transmission of infection. Cannibalism is practiced by lemmings, particularly in years where their number is high [3]. When lemmings take over old nests (for permanent residence or as a temporary shelter), there can be contact with previously infected substrate and further spread of the pathogen. Finally, we should not rule out the role of the transmissive factor in transmission of the pathogen. We have data referable to bacterioscopic detection of Franciselia tularensis in a suspension of Haemogamasus ambulans and Hirstionyssus isabellinus ticks caught in lemming nests. Transmissive infection of young animals by old ones in the nest is particularly likely. For the time being, it is difficult to assess the role of bloodsucking mosquitoes in circulation of the pathogen, since bacteriological examination thereof did not yield positive results.

Conclusions

1. Isolation of F. tularensis from live lemmings, fresh carcasses thereof and water, as well as demonstration of specific antigen in bird pellets and mummified lemming carcasses, are indicative of the existence of independent endemic sites of tularemia of the tundra type.

2. The coincidence of extensive tularemia epizootics with a peak in lemming population size and drastic reduction in population the following year are indicative of the role of epizootics of this disease in reducing the number of animals.

3. Water is the most probable medium where F. tularensis is preserved in periods between epizootics.

4. Apparently, lemmings are infected with F. tularensis through water; infection is possible as a result of cannibalism, as well as through nest-burrow gamasid ticks and the nest substrate.

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# EXPLORATORY INSPECTION OF ENDEMIC SITES FOR TULAREMIA AND PLAGUE IN ARMENIA BY SEROLOGICAL TESTING OF BIRD PELLETS AND EXCRETA OF PREDATORY MAMMALS

Moscow ZHURNAL MIKROBIOLOGII, EPIDEMIOLOGII I IMMUNOBIOLOGII in Russian No 2, Feb 78 (manuscript received 4 Feb 77) pp 111-115

[Article by B. P. Dobrokhotov, A. G. Mnatsakanyan, I. S. Meshcheryakova and M. M. Rudnev, Institute of Epidemiology and Microbiology imeni Gamaleya (Moscow), Armenian Plague-Control Station (Yerevan) and Plague-Control Institute of the Caucasus and Transcaucasia, Stavropol]

[Text] The geographic location, complex mountainous topography and diversity of the environment of Armenia are instrumental in the existence on its territory of endemic sites of various infections, which include widespread sites of tularemia and plague. Tularemia, which was first detected in 1949, is now known to exist in 33 out of the 36 rayons of this republic [6, 7, 9]. It has been established that there are meadow-field, steppe, forest, backwater-swamp and foothill-brook sites. According to the results of many years of bacteriological studies, the pathogen circulates the most often among common voles, less among Transcaucasian hamsters and water rats with the involvement of Ixodes ticks [9]. As for plague, most of Armenia is part of the Transcaucasian high-altitude site of this infection, within which three persistent epizootic districts were singled out: Leninakan, near Lake Sevan and Zangezur-Karabakh [1, 11]. Plague epizootics have been recorded almost annually since 1958. Onset and development of these epizootics are related to the common vole and its specific fleas, and the vole variety of Y. pestis is the pathogen.

Epizootiological observations of tularemia and plague sites have been pursued by the Armenian Plague-Control Station and its departments, as well as the sanitary and epidemiological station, for many years, and this enabled us to use this territory to test the exploratory ["reconnoitering"] method, which was developed in recent years, for detection of epizootics by means of demonstrating antigens of F. tularensis and Y. pestis in bird pellets and droppings of predatory mammals [2, 3, 5].

Between 12 September and 2 October 1974, we inspected northwestern and central regions of Armenia. Our itinerary crossed the Amasiyskiy, Gukasyanskiy, Kalininskiy, Stepavanskiy, Spitakskiy, Akhuryanskiy, Artikskiy, Aparanskiy, Talinskiy, Ashtarakskiy, Razdanskiy, Abovyanskiy, im Kamo, Martuninskiy and Vardenisskiy Rayons. Traveling by motor vehicle, in 14 days 2 people collected in these areas 1973 bird pellets, 34 samples of excrements of wild predatory mammals and 52 rodent carcasses brought in by birds. Along the valleys between mountains, which have been developed by man and are typical of this locality, pellets were collected under communication and power line poles, and in the mountains, near rocky cliffs, exposed rocks at the peaks and triangulation towers. As a rule, dried rodent carcasses abandoned by birds near landing [alighting] places and excrements of predatory mammals were also found in the same places.

The material was tested concurrently for tularemia and plague. For demonstration of antigens of F. tularensis and fraction I of Y. pestis, we used only the antibody neutralization reaction (ANR) with standard tularemia antigenic and plague erythrocytic diagnosticums prepared at the Institute of Epidemiology and Microbiology imeni Gamaleya and the Central Asian Plague-Control Institute. We became convinced previously that the passive hemagglutination reaction with erythrocytes loaded with γ-globulins to Y. pestis and F. tularensis was not suitable for demonstration of antigens in bird pellets, in spite of its simplicity. We used a Takacs microtitrator and conventional methods for preparing specimens and running the ANR [4].

We performed our work independently of the annual scheduled inspection of endemic sites, which is made by the plague-control and sanitary-epidemiological stations, and thus we were able to compare the results (Tables 1 and 2).

Table 1. Results of bacteriological examination of small mammals and their ectoparasites for tularemia and plague in Armenia in 1974

| Object tested | Tularemia | | | Plague | | |
|---|---|---|---|---|---|---|
| | number of specimens examined | specimens with antigen | | number of specimens examined | specimens with antigen | |
| | | abs. | % | | abs. | % |
| Small mammals | 55 710 | 32 | 0,06 | 73 572 | 135 | 0,18 |
| Fleas | 71 061 | 2 | 0,003 | 287 576 | 478 | 0,17 |
| Ixodes ticks | 3 814 | 7 | 0,18 | 6 264 | — | — |
| Gamasid ticks | 86 890 | — | — | 168 752 | 6 | 0,004 |

Table 2. Results of demonstration of antigens of F. tularensis and Y. pestis by the ANR in field specimens collected in an exploratory inspection of sites in Armenia in 1974

| Tested object | Tularemia | | | Plague | | |
|---|---|---|---|---|---|---|
| | total specimens examined | specimens with antigen | | total specimens examined | specimens with antigen | |
| | | abs. | % | | abs. | % |
| Bird pellets | 1973 | 73 | 3,7 | 1973 | 42 | 2,1 |
| Predatory mammal excrements | 34 | 3 | 8,8 | 34 | 1 | 2,9 |
| Mummified rodent carcasses | 52 | 8 | 15,4 | 52 | 5 | 9,6 |

Of the Y. pestis strains isolated from mammals in 1974, 122 (90.5%) were obtained from common voles, 7 from Persian jirds, 4 from an Asia Minor suslik and 2 from weasels; F. tularensis was also isolated the most often from common voles--31 (97%) and only 1 strain from a shrew carcass. These data, as well as the sites where pathogen cultures were isolated from fleas, Ixodes and gamasid ticks were entered on map-diagrams (Figures 1 and 2) and served as a sort of check map that we were to confirm by means of the exploratory inspection.

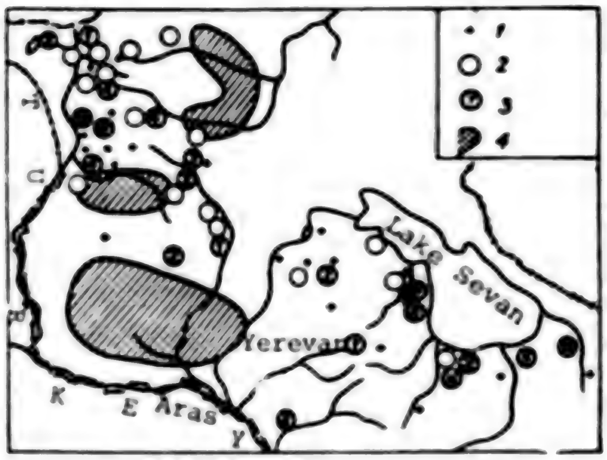

Figure 1.
Tularemia epizootics in Armenia in 1974
1) sites of isolation of F. tularensis strains from mammals, Ixodes ticks and fleas
2) sites of collection of pellets and excrements of predatory mammals
3) points, in which F. tularensis antigen was demonstrated (the numeral shows percentage of positive tests)
4) area inspected, but no pellets were found

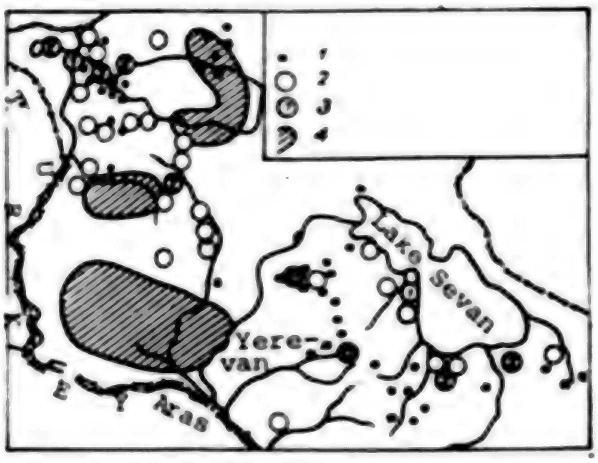

Figure 2.
Plague epizootics in Armenia in 1974
1) sites of isolation of Y. pestis from mammals, fleas and gamasid ticks
2) sites of collection of pellets and excrements of predatory mammals
3) points at which Y. pestis antigen was demonstrated (the numeral shows percentage of positive tests)
4) area inspected, but no pellets found

Material was collected at 37 points, and in 20 of them positive results were obtained in tests for tularemia, 10 in tests for plague. The sites of these pellets coincided rather well with the boundaries of the epizootic territory, and they enabled us to assess its dimensions in 1974. We could not collect any bird pellets only in places where there were very few rodents. There were few specimens with antigen in each inspected point: F. tularensis antigen was presented in 10-17% of only three of these points, and in two points 13-15% of samples revealed fraction I of Y. pestis. At other points, the incidence of positive tests on pellets did not exceed 1-8%. This warranted the assumption that there was a low intensity of tularemia and plague epizootics in 1974 over most of the territory of Armenia. At five points, we found both F. tularensis and Y. pestis antigens. The existence of simultaneous epizootics of these infections in the same places had already been reported in Armenia [8, 10]. We succeeded in demonstrating simultaneous infection with both pathogens in the same animals. Antigen of both F. tularensis and Y. pestis was contained in the remains of two common voles (in 2 pellets) and two mummified common vole carcasses. In the neutralization reaction, the titers of tularemia antibodies of these 4 specimens constituted 1:20 and 1:12, and plague antibody titers were 1:40, 1:128, 1:80 and 1:24.

The amount of antigen in bird pellets can be estimated from the ANR titer. Presence of pellets in the environment leads to gradual flushing out of the antigen with atmospheric precipitations, so that one can tentatively estimate the time of the epizootic. In this case, the mean ANR titer ranged in different points from 1:12 to 1:128 with tularemia antigen and from 1:12 to 1:1428 with plague antigen (maximum titers were 1:512 and 1:2560, respectively). Thus, we detected both epizootics occurring at the time of our inspection and those occurring several months previously.

Analysis of the contents of pellets, in which pathogen antigen was found, revealed (Table 3) that Y. pestis circulates almost exclusively among common voles, whereas F. tularensis circulates among common voles (73.4%) and golden hamsters (19%). We found fraction I of Y. pestis in 5 out of 52 common vole carcasses, whereas F. tularensis antigen was found in 7 common voles and 1 golden hamster. This is consistent with the findings of many years of observation of local sites--common voles are the main hosts of Y. pestis in the Transcaucasian high-altitude site [11]; of the 410 strains of F. tularensis isolated from mammals in 1968-1972, 374 (91.2%) were obtained from common voles, 21 (5.1%) from golden hamsters and only 11 (2.7%) from water rats [6]. It can be assumed that in Armenia there is prevalence of a distinctive variant of a steppe site for tularemia, with very limited distribution of other types of sites.

Table 3. Results of analysis of contents of bird pellets, in which F. tularensis and Y. pestis antigens were found

| Food constituents | Demonstration in pellets of | | | |
|---|---|---|---|---|
| | tularemia antigen (69 pellets)* | | plague antigen (42 pellets) | |
| | absolute | % | absolute | % |
| Mammals: | | | | |
| total** | 105 | 100 | 58 | 100 |
| common vole | 77 | 73.4 | 55 | 94.8 |
| golden hamster | 20 | 19.0 | 1 | 1.7 |
| gray hamster | 2 | 1.9 | — | — |
| water rat | 1 | 1.0 | — | — |
| unidentified species*** | 5 | 4.7 | 2 | 3.5 |

*Contents of 4 pellets were not analyzed.

**Together with the remains of mammals, 3 pellets contained remains of small birds, 7 contained remains of insects and 1 those of a lizard.

***This includes 1 specimen of Apodemus sylvaticus and 1 specimen of Mustela erminea.

The question of advantage of examining bird pellets and droppings of predatory mammals, as compared to ordinary bacteriological methods, was of interest. The probability of detecting antigens of the pathogens of tularemia and plague in this material increases substantially, due to the fact that birds and predators select sick and weakened animals out of the wild animal population, or else pick up their carcasses. The data we obtained enabled us to tentatively assess the efficacy of such "selection." In the 111 pellets analyzed, there were remains of 163 animals, i.e., 1.5 per pellet. Accordingly, in all 1973 collected pellets, there were at least 2959 animals, of which 73 had had tularemia and 42 plague. Accordingly, there was 1 positive pellet per 41 and 70 animals captured by the birds. Bacteriological examination revealed 1 F. tularensis culture in 1741 trapped animals and 1 Y. pestis culture in 545 trapped animals (see Table 1). Thus, the effectiveness of serological testing of animal residue in bird pellets was 42 times greater with respect to tularemia and 7.7. times greater with respect to plague. The difference is apparently attributable to the distinctions of the infectious process in common voles, in whom tularemia is always acute and causes death of these animals, whereas in the case of plague chronic forms are also observed, with which the sick animals are less accessible to birds. Similar findings were made when the ANR was used on carcasses of animals that were not completely consumed by birds and abandoned on their resting sites: 1 finding of tularemia antigen was referable to 6.5 such mummified carcasses and 1 plague antigen per 10.4 carcasses.

## Conclusions

1. Use, for the purpose of an exploratory inspection of tularemia and plague sites in Armenia, of the method of demonstrating pathogen antigen in bird pellets and excrements of predatory mammals made it possible to define rather accurately the size of the territory involved in epizootics, intensity of the latter and animal species involved in the epizootic process.

2. A significant part of the Armenian highlands has mixed and conjugated sites of plague and tularemia, epizootics of which in 1974 often occurred on the same territory and occasionally in the same common voles.

3. Serological testing with the antibody neutralization reaction of remains of small mammals from fish pellets was 42 times more effective for tularemia and 7.7 times more effective for plague than bacteriological examination of animals caught in traps during a zoological inspection of the territory.

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# SOME PATTERNS OF CHANGE IN NUMBER OF CARRIERS AND EPIZOOTIC ACTIVITY IN A LOCAL BACKWATER ENDEMIC SITE FOR TULAREMIA IN VORONEZH OBLAST

Moscow ZHURNAL MIKROBIOLOGII, EPIDEMIOLOGII I IMMUNOBIOLOGII in Russian No 5, May 78 (manuscript received 26 Jul 77) pp 133-138

[Article by V. R. Krasil'nikov and V. S. Sil'chenko, Oblast Sanitary and Epidemiological Station, Voronezh]

[Text] Dedicated to the discoverers of tularemia in the USSR: S. V. Suvorov, A. A. Vol'ferts and M. M. Voronkova.

The Povorinsk endemic tularemia site has long drawn the attention of researchers [7, 8, 12-14] as a classic example of a local backwater site. It is located in the northeastern part of this oblast, in the floodplain of the Khoper and Svintsovka Rivers. It covers an area of more than 6000 ha. The location of this site on the boundary between forest-steppe and steppe regions provides for a significant abundance and diversity of fauna [11, 12]. It is inhabited by more than 35 species of rodents, insectivores and carnivores [or predatory mammals] [1]. The most frequently encountered species are the water and common redbacked [or bank] voles, common and striped field mice, and the house mouse. The yellow-necked field mouse, beaver, common shrew, water-shrew and muskrat [or desman] are common to these areas. Bloodsucking insects are widely represented by horseflies, mosquitoes, biting midges and gnats. Ixodes ticks are represented by an insignificant number of Ixodes ricinus and Dermacentor marginatus.

Epizootic and epidemiological observations have been pursued of this site since 1930,* but they started to be systematic and scheduled in 1958. In the period from 1958 to 1974, 19,326 mammals (Table 1), 98,700 bloodsucking Diptera, 408 Ixodes ticks and 1672 water samples were examined. As a result, 370 cultures of F. tularensis were isolated, of which 352 were from the water vole, 4 from the striped field mouse, 1 from water-shrew and 13 from water. No cultures were isolated from ticks.

As a result of studies of the dynamics in rodent population size and epizootic activity between 1958 and 1974, it was possible to divide this time into two periods. The first period (1958-1968) was characterized by a large population of water voles (35-88% filled traps along the shoreline during the spring flood tide), while the number of other rodents held at a constant low level (maximum trapped 16.5%). The second period (1969-1973) began with an unusually rigorous winter without snow, which caused, along with drying of swamps, a severe depression of the water vole population lasting to the end of this period; the number of small rodents was rapidly restored and reached a high level by the summer of 1970 (50-70% of traps had animals).

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*Ye. G. Reznikov, S. A. Rastorguyeva, A. S. Sokol'skaya, A. V. Rayskaya, V. G. Klenova, T. D. Markova, B. G. Chebotarev, Ye. N. Yezhova, L. M. Grishayev, M. P. Lysenko and others.

Table 1. Species composition and quantity of animals examined

| Species | Quantity of animals | | Cultures isolated | |
|---|---|---|---|---|
| | absol. | % | abs. | % |
| Water vole | 17 654 | 91,35 | 352 | 98,6 |
| Common redbacked vole | 574 | 2,97 | — | — |
| Common vole | 67 | 0,35 | — | — |
| Striped field mouse | 707 | 3,66 | 4 | 1,1 |
| Common field mouse | 132 | 0,68 | — | — |
| Yellow-necked field-mouse | 44 | 0,23 | — | — |
| House mouse | 52 | 0,26 | — | — |
| Common shrew | 73 | 0,38 | — | — |
| Water-shrew | 7 | 0,04 | 1 | 0,3 |
| Other mammalian species | 16 | 0,08 | — | — |
| Totals | 19 326 | 100,0 | 357 | 100,0 |

It was established that all of the epizootics in this site were referable to a period of large number of water voles, i.e., up to the winter of 1968-1969. There was very negligible involvement of other rodents in the tularemia epizootic, 1.4% of total cultures isolated. We failed to demonstrate any tularemia epizootics from 1969 on, when there was a large number of small rodents (Figure 1).

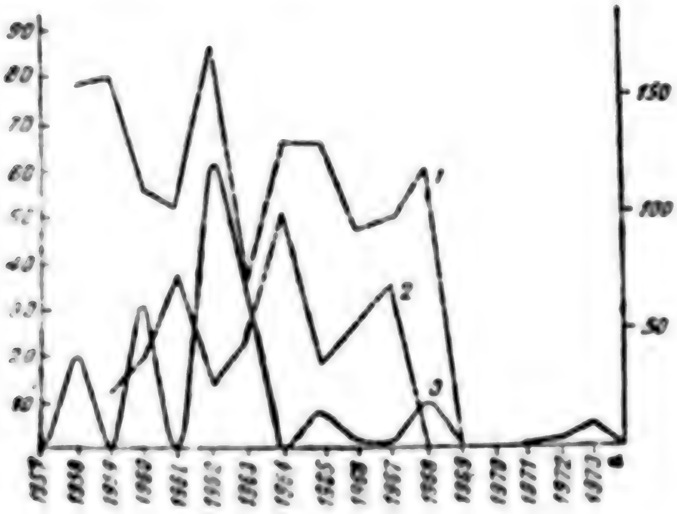

Figure 1.
Dynamics of water vole population size and intensity of tularemia epizootics in the Povorinsk endemic site

1) number of water voles in the spring (flood tide)
2) fall
3) intensity of epizootics

Y-axis: left, % caught in traps; right, number of cultures isolated per 1000 tested animals

The results of further investigation of epizootic activity at the Povorinsk backwater endemic site for tularemia warranted consideration thereof as being of the single-host type [10]. Epizootic outbreaks were observed mainly among water voles, and they were the cause of the epizootic problem in this site [4].

Apparently, there must be a minimum (threshold) number of water voles, which are the main carrier of F. tularensis, for epizootics to occur. In the Povorinsk enzootic site, this level corresponded to about 30% trapped water voles. It should be thought that this was close to the threshold level.

Table 2. Quantity of animals tested annually

| Year | Water vole | | | Other species | | | Total animals tested | Cultures isolated from water | Number of cultures | Cultures/ 1000 tested animals |
|---|---|---|---|---|---|---|---|---|---|---|
| | number of tests | % trapped | number of cultures | number of tests | % trapped | number of cultures | | | | |
| 1958 | 1 270 | 80,0 | 4 | 23 | — | — | 1 293 | — | 4 | 3,1 |
| 1959 | 1 997 | 80,5 | — | 110 | 16,5 | — | 2 107 | — | — | — |
| 1960 | 1 030 | 56,7 | 67 | 71 | 10,4 | — | 1 101 | — | 67 | 61 |
| 1961 | 1 262 | 51,7 | — | 34 | 10,3 | — | 1 296 | — | — | — |
| 1962 | 1 184 | 88,5 | 167 | 185 | 15,7 | 2 | 1 369 | 2 | 171 | 123 |
| 1963 | 1 011 | 35,3 | 52 | 68 | 18,5 | — | 1 079 | 4 | 56 | 48 |
| 1964 | 1 005 | 66,6 | — | 16 | 5,5 | — | 1 021 | — | — | — |
| 1965 | 2 172 | 65,5 | 31 | 47 | 9,3 | — | 2 219 | 4 | 35 | 14 |
| 1966 | 2 358 | 46,3 | 4 | 229 | 10,3 | 1 | 2 587 | — | 5 | 1,9 |
| 1967 | 2 868 | 49,1 | 1 | 156 | 11,7 | 2 | 3 024 | — | 3 | 0,9 |
| 1968 | 1 478 | 60,1 | 26 | 51 | 9,7 | — | 1 529 | 3 | 29 | 17 |
| 1969 | — | — | — | 42 | 7,8 | — | 42 | — | — | — |
| 1970 | 1 | 0,5 | — | 298 | 49,2 | — | 299 | — | — | — |
| 1971 | — | — | — | 146 | 50 | — | 146 | — | — | — |
| 1972 | — | — | — | 14 | 16 | — | 14 | — | — | — |
| 1973 | 16 | 2,0 | — | 83 | 216 | — | 99 | — | — | — |
| 1974 | 2 | 2,0 | — | 99 | 22,5 | — | 101 | — | — | — |
| Totals | 17 654 | — | 352 | 1672 | — | 5 | 19 326 | 13 | 370 | — |

From 1958 to 1968, when the number of water voles was consistently above the threshold level, tularemia epizootics were recorded almost annually. However, their intensity varied, and it was unrelated to fluctuations in number of carriers. Thus, the 1958 epizootic occured with an 80% level of trapped animals, whereas in the spring of 1959, when there were somewhat more water voles, no epizootic was found, although more animals had been examined than in 1958 (Table 2). By the spring of 1960, the incidence of trapping animals dropped to 56% and, in spite of this, there was a tularemia epizootic (61 cultures of F. tularensis per 1000 tested animals). Water voles multiplied intensively in 1961 (52% trapped), but there was no epizootic.

In 1962, the maximum number of water voles for the entire observation period was recorded, and the most intensive epizootic was demonstrated among them (123 cultures per 1000 examined animals). The epizootic continued in 1963, but it did not affect the number of water voles, which reproduced intensively, and by the spring of 1964 the incidence of trapping them reached 66%, but no epizootic occurred that year. In 1965-1967, the dynamics of intensity of epizootics were identical to 1962-1964, but the epizootics were not as acute.

Table 3.
Incidence of tularemia among people and isolation of F. tularensis at the Povorinsk site

| Year | Number of sick cases | Number of cultures isolated | Year | Number of sick cases | Cultures isolated |
|---|---|---|---|---|---|
| 1930 | 2 | — | 1951 | 4 | 1 |
| 1934 | 103 | — | 1952 | 3 | 2 |
| 1936 | 32 | — | 1953 | 1 | — |
| 1938 | 183 | 37 | 1958 | 1 | 3 |
| 1939 | 6 | 2 | 1959 | — | 4 |
| 1941 | 33 | — | 1960 | 1 | 67 |
| 1943 | 377 | — | 1962 | 2 | 171 |
| 1945 | 1481 | 3 | 1963 | 1 | 56 |
| 1946 | 347 | 3 | 1965 | — | 35 |
| 1947 | 1 | — | 1966 | — | 5 |
| 1948 | 10 | 6 | 1967 | — | 3 |
| 1949 | 62 | — | 1968 | — | 29 |
| 1950 | 5 | — | 1969—1974 | — | — |

Notes:

1. There were no cases of tularemia among people and no cultures isolated in years not listed here.
2. In 1943 and 1945-1946, cases of tularemia among people were related to a tularemia epizootic among mouse-like rodents--common voles and house mice.

Thus, from 1958 to 1968 the intensity of epizootics changed drastically. It increased from 1958 to 1962 and decreased by 1968. The rise and decline were not uniform, presenting a wavy pattern with 2-3-year period. The phase of growth in intensity consisted of 2 waves with a 2-year period (1958-1959, 1960-1961). The phase of decline consisted of 2-3-year waves (1962-1964, 1965-1967). The entire cycle covered 10 years.

Long-term observation of water vole population size enabled us to conclude that the tularemia epizootics at the Povorinsk site definitely had an influence on it, but it was not always the same and depended on the phase (rise, decline) of epizootic activity. At the phase of rise, when the epizootics had a 2-year period, the number of water voles dropped significantly (1958, 1960); at the phase of decline in intensity of epizootics, which occurred in 3-year waves, the number of water voles dropped drastically (1962, 1965), whereas the second year, in spite of the continuing epizootic, the number of animals was restored (1963, 1966).

The tularemia epizootics, which caused considerable fluctuation in number of water voles in different years [14], could not elicit a lasting decline thereof. A lasting decline was caused by abiotic factors and anthropogenic factors [5, 6, 9]. When the number of water voles dropped below the threshold level, this site moved to an inactive epizootic phase. Kalabukhov [2], Karpov [3] and Kondrashkin [4] arrived at the same conclusion with regard to backwater sites.

We analyzed data on incidence of tularemia among people who had been in the range of the Povorinsk site (hunting for water voles, water use, agriculture, etc.) in order to characterize more fully the changes in epizootic activity of this site. Our findings revealed (Table 3) that there was significant variation of morbidity in different years (Figure 2): years with no tularemia problem were followed by years with marked incidence of the disease among people. We were impressed by the coincidence of sequence of periods of epizootic activity and epidemic well-being. Here too, we observed rises and extinctions of infections, the same cycles and waves as with the epizootics. From 1933 to 1939, one 3-year and two 2-year waves of morbidity were recorded, and the entire cycle covered 7 years. From 1940 to 1949, the 10-year cycle included two 2-year waves. Significant changes have been occurring since 1946: drastic decrease in outbreaks of tularemia with further change to isolated sporadic cases of disease. That year marked the beginning of systematically implemented scheduled preventive immunization against tularemia.

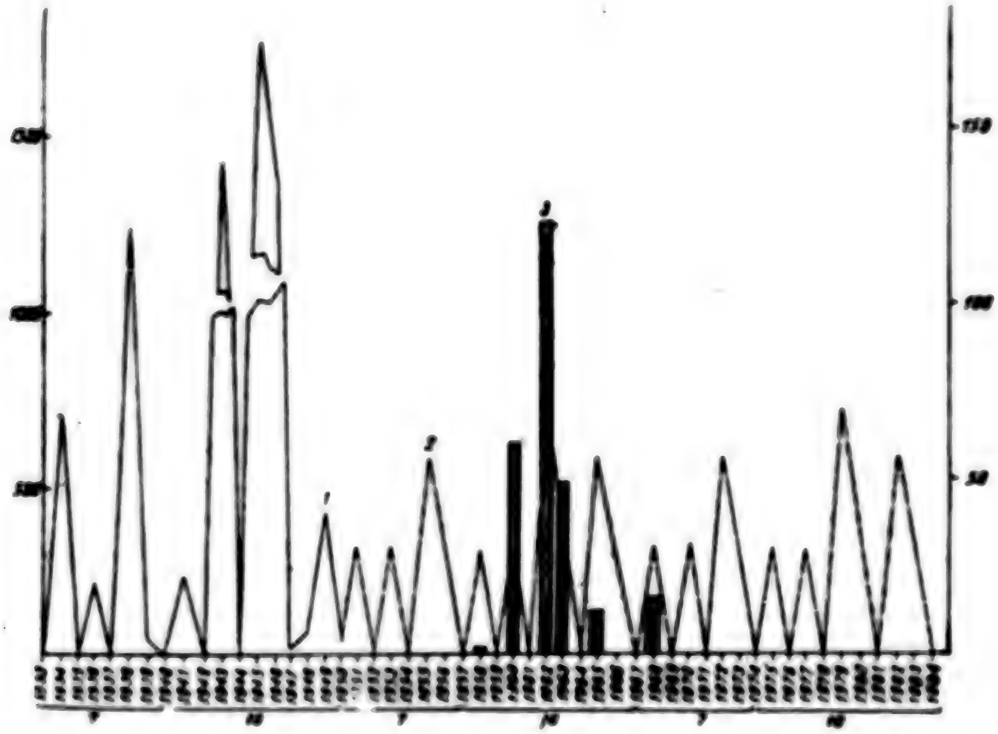

Figure 2. Incidence of tularemia in Povorinskiy Rayon and intensity of epizootics. X-axis, years; y-axis: left, number of sick cases per 100,000 population; right, number of cultures isolated per 1000 tested animals

1) number of sick cases per 100,000 population
2) estimated morbidity curve
3) number of cultures isolated per 1000 tested animals

If we assume that there is alternation of 7- and 10-year cycles and, on this basis, plot a hypothetical morbidity curve (it is also the epizootic curve), we would find that since 1958 it coincides entirely with the actual curve of epizootic activity. An increase in number of water voles started in 1974; tularemia epizootics were demonstrated among them in 1975 and 1977, which corresponded with our projected curve of epizootic activity. Investigation and demonstration of the distinctions referable to dynamics of tularemia epizootics, their influence on the number of voles, which are carriers of this infection, make it possible to produce a long-term forecast and plan epidemic control measures for endemic tularemia sites in backwater-swamp regions.

## Conclusions

1. In a backwater endemic site in Povorinsk, tularemia epizootics occurred when there was a large number of water voles; other rodents and Ixodes ticks did not play an appreciable role in maintaining endemicity of the infection.

2. The threshold number of water voles, which presented a threat of a tularemia epizootic constituted a 30% incidence of trapping the animals; if the number was above the threshold level, the fluctuations in epizootic activity at the site did not depend on changes in number of carriers.

3. Epizootic activity at this site was characterized by alternation of 7- and 10-year cycles, which consisted of a certain number of waves occurring with 2-3-year periods; at the phase of rise in intensity (2-year waves) of epizootics, there was significant decrease in number of water voles, at the phase of decline (3-year waves), the number of animals dropped drastically the first year and rose the second year, in spite of the epizootic.

4. Onset of tularemia among people at the site usually coincided with periods of high epizootic activity in water voles.

5. The demonstrated distinctions of dynamics of epizootic activity and its influence on number of the main carriers of tularemia infection made it possible to formulate a long-term forecast and plan epidemic-control measures.

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# SENSITIVITY OF SOME F. TULARENSIS STRAINS OF THE HOLARCTIC RACE ISOLATED IN KAZAKH SSR TO DIFFERENTIAL DOSE OF ERYTHROMYCIN AND OLEANDOMYCIN

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[Article by O. B. Chimirov, Central Asian Scientific Research Plague-Control Institute, Alma-Ata]

[Text] Determination of sensitivity to erythromycin and oleandomycin of different strains of F. tularensis is an additional test for differentiation of its geographic races [3, 5]. This test is based on the high resistance of the Holarctic race of the pathogen of tularemia to erythromycin (100 μg/mℓ or more) and oleandomycin (400 μg/mℓ or more), whereas strains of the Central Asian and Nearctic race of F. tularensis are sensitive to the above-mentioned doses of these antibiotics [1, 3, 5].

Previously [3], strains of the Holarctic race of this microorganism, which were isolated in the USSR, were characterized only by resistance to erythromycin and oleandomycin, and for this reason this trait served as an additional differential test. However, recent works [2, 4] established that some strains of the Holarctic race isolated in Taymyr, Buryat ASSR, Yakutsk ASSR, Chita and Khabarovsk Oblasts, Maritime Kray and Sakhalin, as well as some strains isolated in Italy, France, Sweden, China and Japan, were found to be sensitive to erythromycin and oleandomycin.

Our study of a number of strains of the Holarctic race of F. tularensis isolated in 1973-1975 in Kazakh SSR revealed that not all of the strains were resistant to differential doses of erythromycin and oleandomycin. For this reason, we decided to determine the degree of sensitivity of F. tularensis of the Holarctic race to these macrolides.

We took 33 strains of F. tularensis isolated in Kazakh SSR in 1973-1975 for our experiment; 6 of them were isolated from Turgay Oblast, 18 from Taldy-Kurgan Oblast and 9 from Uralsk Oblast. All of them presented typical morphological, cultural and biochemical properties; they did not decompose glycerin on Downs' medium and had no citrulline ureidase activity [6], i.e., they were referable to the Holarctic race and caused death of white mice given hypodermic injections of isolated bacterial cells.

Experiments were conducted on Heddleson's liver-glucose-glycerin-cysteine agar with addition of 10% defibrinated rabbit blood at pH 7.2. The antibiotics were diluted in distilled water and specific doses (100 μg/mℓ erythromycin or 400 μg/mℓ oleandomycin) were added to dissolved agar cooled to 45°C at the same time as defibrinated blood. After thorough mixing, the medium was decanted in Petri dishes and kept for 1 day at room temperature.

Table 1. Behavior of tested strains with erythromycin (E) and oleandomycin (O)

| Where strains were isolated | Strains tested | Number of strains resistant to E & O | Number of strains sensitive to E & O |
|---|---|---|---|
| Turgay Oblast | 6 | 5 | 1 |
| Taldy-Kurgan Oblast | 18 | 10 | 8 |
| Uralsk Oblast | 9 | 9 | - |
| Totals | 33 | 24 | 9 |

In the experiments, we used a suspension of 1 billion bacterial cells per mℓ saline (according to the enteric standard of GKI [State Control Institute of Medical Biologicals]), prepared just prior to inoculation on media with antibiotics from a day-old culture of F. tularensis grown at 37°C in Heddleson's agar. We plated 4 strains in each Petri dish containing medium with antibiotics, one of which was known to be resistant to the antibiotic in question,a and was a typical representative of the Holarctic race (strain No 263), another was a typical representative of the Central Asian race of F. tularensis sensitive to these antibiotics (strain No 31).

Using a 1-mℓ pipette, we inoculated 0.05 mℓ, which corresponded to 50 million bacterial cells. The same suspension was used to inoculate a control Petri dish with medium containing no antibiotic. The cultures were incubated for 10 days at 37°C and examined daily. We assessed sensitivity or resistance of the strains to antibiotics on the basis of appearance or absence of growth of F. tularensis on this medium, as well as in comparison to bacterial growth in the control dish. Concurrently, we examined under a microscope smears of cultures that grew in medium with antibiotic, which were stained by the Gram method.

The results of this experiment revealed (Table 1) that there were strains that were both resistant and sensitive to the tested doses of antibiotics among the examined cultures. We failed to demonstrate a difference in virulence of resistant and sensitive strains of F. tularemia of the Holarctic race for white mice--all of the animals died after hypodermic infection with isolated bacterial cells.

In determining the degree of sensitivity to erythromycin and oleandomycin, we found (Table 2) that the minimum inhibitory concentration (MIC) of antibiotics for these strains was considerably lower than the differential dose of erythromycin (100 μg/mℓ) and oleandomycin (400 μg/mℓ). As to other antibiotics, all of the strains were highly resistant to penicillin (MIC = 1000 μg/mℓ) and polymyxin (MIC = 2000 μg/mℓ) and sensitive to tetracycline (MIC = 5 μg/mℓ) and streptomycin (MIC = 2.5 μg/mℓ).

We failed to demonstrate any correlation between reaction to erythromycin, oleandomycin and biochemical activity of the tested cultures, as well as their source: we found strains that were both sensitive and resistant to what was previously considered differential doses of these macrolides among strains that did not decompose glycerin and did not have citrulline ureidase activity.

Table 2. Characteristics of strains of F. tularensis holarctica sensitive to differential doses of erythromycin and oleandomycin

| Strain No | Source | Date of isolation | MIC, μg/mℓ | |
|---|---|---|---|---|
| | | | erythro-mycin | oleando-mycin |
| 205 | Ar. terrestris | 27/VIII 1973 | 6 | 12 |
| 316 | D. marginatus | 22/VI 1975 | 12 | 100 |
| 332 | D. marginatus | 23/VI 1975 | 6 | 100 |
| 333 | D. marginatus | 23/VI 1975 | 6 | 100 |
| 334 | D. marginatus | 25/VI 1975 | 6 | 50 |
| 344 | D. marginatus | 20/VI 1975 | 6 | 50 |
| 355 | D. marginatus | 20/VI 1975 | 6 | 50 |
| 356 | D. marginatus | 20/VI 1975 | 6 | 50 |
| 358 | D. marginatus | 20/VI 1975 | 6 | 50 |

Thus, the demonstrated difference in response to differential doses of erythromycin and oleandomycin of strains of the Holarctic race of F. tularensis, which were isolated on the territory of Kazakh SSR, confirmed the data of Brikman et al. [2] to the effect that this test is not suitable for differentiating between geographic races of the pathogen of tularemia.

## Conclusions

1. Among strains of the Holarctic race of F. tularensis, which were isolated in Kazakh SSR, some were sensitive and others resistant to erythromycin and oleandomycin.

2. Sensitivity of the bacteria to erythromycin and oleandomycin was unrelated to the source of the pathogen or to virulence and biochemical properties of strains.

3. The presence of strains of the Holarctic race of F. tularensis, which were both resistant and sensitive to differential doses of erythromycin and oleandomycin, on the territory of Kazakh SSR makes it impossible to differentiate between their geographic races according to this property.

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# ENDEMIC INFECTIONS IN THE NORTHERN PART OF THE FAR EAST. REPORT 1: DETECTION OF ENDEMIC SITES IN CHUKOTKA

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[Article by G. P. Somov, F. N. Shubia, I. M. Gopachenko, G. N. Leonova, T. P. Ivanenko, S. A. Sorochenko, A. N. Sotnikova, V. G. Kuznetsov and S. P. Kruglyak, Vladivostok Institute of Epidemiology and Microbiology, Siberian Branch of the USSR Academy of Medical Sciences, Novosibirsk; Maritime Kray Plague-Control Station, Ussuriysk]

[Text] The rapid industrial development of the northern part of the Far East, because of the exceptional wealth of natural resources in that region, has advanced several biomedical problems aimed at improving man's life under extreme conditions. The question of presence of endemic sites of infection in the vast territory of the northern Far East, about the existence of which virtually nothing was known until very recently, is of some importance among these problems.

The major outbreaks of Far Eastern scarlatinoid fever (human pseudotuberculosis in Chukotka [Chukotsk Peninsula] drew attention to the study of endemic diseases in the northern Far East [1, 3, 6]. The results of serological studies pursued in this regard revealed that there were antibodies to the pathogens of tularemia, leptospirosus, pseudotuberculosis, brucellosis, ornithosis, toxoplasmosis and Q fever in the blood of people and animals [4, 5].

A many-year complex program was elaborated for the study of endemic infections in the northern Far East in order to resolve the problem that emerged; implementation thereof was started in 1974 in the form of organizing annual expeditions. We submit here some of the results of 3-year studies, the purpose of which was to find endemic sites of some infections in Chukotka, investigation of their structure and mechanisms of circulation of pathogens under the rigorous climate and geographic conditions of the North.

We first made a serological study of 1027 reindeer with antigens of Y. pseudotuberculosis and Yersinia enterocolitica using the agglutination reaction, F. tularemia by the indirect hemagglutination reaction, Rickettsia by the CFT [complement fixation test] and arboviral infections by the hemagglutination inhibition reaction. As a result, it was established that antibodies to the pathogen of pseudotuberculosis serotype I were present in 13.3±0.7% of the animals, to serotype III pseudotuberculosis in 10.5±1.03%, to Y. enterocolitica (strain № 443) in 2.92±0.55%, tularemia in 10.58±1.4%, North Asian tick-borne [mite?] typhus in 1.05±0.4%, Q fever in 1.42±0.4% and tick-borne encephalitis in 1.6±0.53% of the tested reindeer. Analysis of 85 samples of blood from Arctic susliks also demonstrated antibodies to serotype I of Y. pseudotuberculosis in 5 animals and serotype III in 10, and when blood from 23 susliks was tested with antigen of tick-borne encephalitis virus antibodies were found in 2 animals.

Thus, the obtained data were indicative, in the first place, of the possibility of existence in Chukotka of endemic sites of these infections and, in the second place, of the possibility of circulation of the pathogens of zooanthroponoses among small mammals and their nest-burrow parasites under the rigorous climate conditions of the Chukotsk tundra, as was demonstrated in Taymyr [2]. Migratory birds, which could be the link between endemic sites of diseases in remote climate and geographic zones, play a part in this process.

The microbiological and virological studies made it possible to confirm in part, the results of serological studies. In all, 268 specimens of mammals were caught and submitted to virological testing in different parts of Chukotka, including 182 Arctic susliks, 46 northern redbacked voles, 21 large-toothed redbacked voles, as well as a a few root voles, brown rats and northern pikas, 95 migratory birds represented by 11 species and the contents of 45 eider nests.

Material from these animals (liver, spleen, segment of intestine) was delivered for tests in a frozen state in insulated containers with liquid nitrogen. For testing for pseudotuberculosis, macerated organs and segments of intestine were plated separately in buffer solution by the method of Paterson and Cook [7]. The cultures were kept at 4°C for 30 days, and periodically subcultured on differential nutrient media.

We isolated 9 strains of Y. pseudotuberculosis from Arctic susliks, thereby establishing, for the first time, spontaneous carriers in this species. In addition, isolation of Y. pseudotuberculosis strains from susliks showed that endemic sites for pseudotuberculosis can exist in the northern part of the Far East which, in turn, enables us to relate outbreaks of this infection to circulation of the pathogen in the environment.

Concurrently, the delivered material was tested by a similar method for Y. enterocolitica. We isolated a total of 48 Y. enterocolitica strains. Most of these strains were homogeneous in biochemical properties. They fermented to acid glucose, maltose, mannitol, saccharose, xylose, sorbitol, did not split lactose, dulcitol, raffinose, did not form hydrogen sulfide, whereas there were both positive and negative variants with regard to production of indole and fermentation of rhamnose. All of the strains were nonmotile at 37°C. At both temperatures, they reacted positively with methyl red and formed acetyl methylcarbinol at 22°C.

Spontaneous Y. enterocolitica carriers were found among the Arctic suslik, brown rat, northern redbacked vole, shrew and six bird species: long-tailed duck, tufted duck ["chernyat'?], Temminck's stint [sandpiper], glaucus gull and alpine ptarmigan.

The incidence of Y. enterocolitica infection is rather high among different animal species. For example, it reached 15.93±2.7% in the Arctic suslik. However, no antibodies to Y. enterocolitica were demonstrated in a test of blood serum from 85 susliks with live cultures of isolated strains and autologous strains.

Material from 238 small mammals and birds caught in the valleys of the Anadyr and Tanyurer Rivers were submitted to bacteriological analysis for salmonellosis. Two strains of Salmonella heidelberg were isolated, which were typical in morphological, biochemical and antigenic properties. Spontaneous Salmonella carriers were found among the northern redbacked voles, thereby demonstrating for the first time the endemicity of this salmonellosis in the extreme northeastern part of Asia.

For demonstration of arboviruses, we examined 237 birds of 20 species, 30 rodents (northern pika, northern redbacked and large-toothed redbacked voles), 63 Arctic susliks and 1 hare. All of the material was analyzed in 97 biotests on suckling mice. We isolated four strains of viruses, which were identified as tick-borne encephalitis virus according to biological and antigenic properties. Spontaneous carriers were found in two bird species (ruff and long-tailed duck), the Arctic suslik and northern redbacked vole. The virus of tick-borne encephalitis was isolated from the ruff in the early spring, which indicates that the virus is carried by birds to the northern regions of the Far East. Isolation of the virus from the Arctic suslik and northern redbacked vole, on the contrary, indicated that there was an endemic site for tick-borne encephalitis in Chukotka. It can be assumed that nest-burrow parasites and, perhaps, mosquitoes are involved in the process of circulation of tick-borne encephalitis virus in the environment of the Extreme North, since the percentage of seropositive results with regard to tick-borne encephalitis virus was about the same when we tested reindeer grazing in different physicogeographic regions of Chukotka (see Table).

Results of serological testing of Chukotka reindeer with antigens of pathogens of some zooanthroponotic diseases (M±m)

| Physicogeographic region | F. tularensis | | Tick-borne encephalitis virus | | Pathogen of Asian tick-borne rickettsiosis | | Pathogen of Q fever | |
|---|---|---|---|---|---|---|---|---|
| | sera | antibodies found,% | sera | antibodies found,% | sera | antibodies % | sera | antibodies % |
| Anyuysk-Chukotsk bald peak and tundra highlands | 302 | 4.0±1.13 | 322 | 2.2±0.82 | — | — | — | — |
| Anadyr-Penza bald peak and forest-tundra plateau | 403 | 9.9±1.48 | 403 | 2.0±0.7 | 302 | 1.7±0.74 | 302 | 2.0±0.81 |
| Koryaksk bald peak and forest-tundra highlands | 139 | 9.4±2.48 | 130 | 1.[illegible]±0.99 | 246 | 0.8±0.57 | 246 | 0.8±0.57 |

Only serological tests were made (see Table) for detection of tularemia and endemic rickettsiosis. Antibodies to the pathogens were found in reindeer from all of the examined physicogeographic regions of Chukotka.

Thus, as a result of these studies in Chukotka, for the first time there was demonstration of endemic sites of pseudotuberculosis, enteric yersiniosis, heidelberg type salmonellosis and tick-borne encephalitis. Two animal species play a large part in circulation of the pathogens of zooanthroponoses, and they are also the most numerous--Arctic suslik and northern redbacked vole--and this creates the conditions for formation of combined sites of infection.

Considering the data on isolation of tick-borne encephalitis virus on Taymyr Peninsula [2] and the results of our studies, it can be concluded that tick-borne encephalitis virus circulates in northern Siberia and Far East. The absence of Ixodes ticks at these latitudes warrants the assumption that there are special routes of circulation of the virus in the environment, and this requires further in-depth investigation.

Conclusions

1. Endemic sites of pseudotuberculosis, enteric yersiniosis, heidelberg type of salmonellosis and tick-borne encephalitis were found for the first time in the subarctic tundra and forest-tundra of Chukotka.

2. Isolation of tick-borne encephalitis virus from the ruff and long-tailed duck during the spring migration was indicative of the possibility that migratory birds brought the virus to Chukotka.

3. The results of serological studies are indicative of the expediency of further investigations for detection of endemic sites for tularemia and endemic rickettsiosis in Chukotka.

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# CURRENT STATUS OF TULAREMIA PREVENTION IN THE USSR AND FUTURE TASKS

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[Article by G. P. Nikolayevskiy, V. I. Frolov and V. I. Morozov]

[Text] The achievements of Soviet health care in the area of preventing infectious diseases are common knowledge. Considerable advances have been made, not only in the area of controlling infections that were widespread in prerevolutionary Russia, such as typhus and relapsing fever, smallpox, cholera, malaria, anthrax and others, but considerable work has also been done on the study and development of a system of measures for the prevention of many diseases that had not been given attention in tsarist Russia.

One of these diseases is tularemia. No doubt, it was encountered in our country since time immemorial; however, the illness in people most often was diagnosed as plague, plague-like disease, fever, etc.

In the years that have passed since the pathogen of tularemia was identified in the USSR (1926), Soviet researchers have accumulated basic material on the microbiology, laboratory diagnosis, pathology, symptomatology, therapy, immunology and prevention of this infection. Soviet researchers made greater advances than foreign authors in the study of many of these areas.*

From 1926 to 1927 (a 9-month period), 561,510 cases were recorded among people in the USSR. Since there was no mandatory registration, which was adopted in 1941-1942, the morbidity records of prewar years were, of course, incomplete.

One can arbitrarily distinguish five periods in the dynamics of tularemia among the people of the USSR. The first period (1926-1932) was referable to active detection tularemia in our coutry; in these years there were 11, 287 cases; the second period (1933-1940) was one of investigation of the infection and development of a system of epidemic-control and preventive measures, and already 40,002 cases of tylaremia were found; considering the absence of mandatory registration of diseases in these years, it can be assumed that the true incidence of tularemia was somewhat higher; the third period (1941-1949) was a period of maximum incidence of the infection among people, and there were

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*Olsuf'yev, N. G., MED. PARAZITOL., No 3, 1977, pp 273-282.

482,413 registered cases. It should be noted that in these years, mandatory registration of tularemia had already been adopted, and this guaranteed a full record of cases in the nation, with the exception of occupied territories.

The wide distribution of tularemia during the war and early postwar years was attributable to failure to adherence to principles of agriculture due to military operations, intensive reproduction of rodents, who were the sources of infection, migration of the public, long-time presence or exposure to endemic tularemia sites. In addition, tularemia vaccine was not yet in use for mass inoculation of the public, and this practice was adopted in public health in 1948-1949.

The fourth period (1950-1964) was characterized by a decisive attack on the infection by means of an effective system of measures, the principal one being mass scale vaccination of the public with live tularemia vaccine in enzootic regions. During these years, there were still 34,280 registered cases of this disease among people. In this period, there was intensive development and completion of the system of measures for tularemia prevention, and the minor outbreaks of tularemia (1954, 1957) were related to development of regions where there were previously unknown endemic tularemia sites.

Thus, the combined ameliorative measures in endemic sites of infection, based on a comprehensive study of tularemia, as well as regular mass-scale immunoprophylaxis of the disease made it possible to reduce the incidence of tularemia to isolated cases, in spite of the poor epizootic situation in the country.

It can be considered that tularemia in the USSR has been a controlled infection since 1965, the fifth period in the dynamics of human morbidity. Since that time, there have been isolated cases of the disease and cases among small groups of unvaccinated individuals (usually not immunized because of medical indications). As a result, 1703 cases of tularemia among humans were recorded in the USSR in 13 years (1965-1977), although the epidemic activity of many enzootic sites of infection was very high. This was indicated by the intensive epizootics among rodents, isolation of F. tularensis cultures annually from both rodents and ticks, as well as environmental objects, mainly water. The advances made in the USSR in the control of tularemia are even more impressive, if we consider that endemic sites of infection are still being found virtually all over the country.

At the same time, the intensive epizootic situation with regard to tularemia in the USSR makes it imperative to implement mandatory and proper tularemia-control measures. When such work is not done properly or sufficiently, group diseases occur. In these years, group diseases constituted more than 90% of all cases of tularemia among people. Isolated, sporadic cases are encountered rather seldom. Most active were endemic sites of the backwater-swamp and mountain (foothill)-brook types, in which more than 97% of the tularemia-stricken people were infected.

As a rule, group cases occurred in sites of the backwater-swamp type in a typical period of the year, summer and fall, particularly July and August. Unimmunized people became ill in endemic tularemia sites, among those engaged in mowing hay, gathering mushrooms and berries, vacationers, swimmers in waters contaminated by excrements of rodents with tularemia. The transmissive and water routes were the principal ones in transmission of infection in these sites.

The cases of group disease in endemic sites of the mountain (foothill)-brook type in Transcaucasian repbulics were distinctive. There, the following causes were involved in infecting humans: water factor of transmission in Armenian SSR, and people became ill in March and September-October; alimentary route (March) in Azerbaijan SSR; in Georgian SSR through ticks when people came in contact with livestock grazed in endemic tularemia sites (October-November).

It should be noted that these group cases were due not only to the activity of some endemic tularemia sites, but flaws and serious infractions of the rules for preventive tularemia-control measures.

Most of the tularemia cases are recorded in the RSFSR (74.7% of all morbidity since 1972), Transcaucasian republics--14.6% and Kazakh SSR--8.5%, with 2.2% for the rest of the regions.

In assessing the measures for tularemia prevention, we must mention that they are combined, and this is reflected in the relevant work plans. At the same time, the principal preventive measures are implement by public health agencies and institutions, unlike other zoonotic infections, for which measures implemented by the USSR Ministry of Agriculture, other ministries and agencies play a large part. With regard to tularemia, other ministries and agencies implement measures for the control of rodents and vectors.

In order to properly organize tularemia-control measures, in the USSR much attention is given to inspection of endemic tularemia sites, exploration of regions where there may be such sites. In our country, considerable territories of endemic sites are inspected annually, up to 400,000 rodents, up to 800,000 bloodsucking arthropods, many samples of water, grain and other material. The method of inspecting sites by means of demonstrating antibodies in bird pellets and droppings of carnivorous mammals is being used more and more.

Epizootics are also monitored and regulated by means of planned control of rodents and vectors, together with concerned ministries and agencies. A total of 30-60 million ha fields and 3-4.5 billion $m^2$ in populated areas are treated annually in our country for eradication of rodents (Table 1).

Table 1. Parameters of treatment of areas against rodents*

| Regions | 1974 | 1975 | 1976 |
|---|---|---|---|
| Fields, thousands of hectares | 37,188 | 31,045 | 62,567 |
| Populated centers, thous. $m^2$ | 4,367,210 | 3,867,389 | 3,241,779 |

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*Not counting the work done by plague-control institutions.

Substantial work is being done in the USSR with regard to inspection of regions in which no endemic sites are known, but they could be present. Such sites are being inspected with particular intensity in regions under development, in particular along the route of the Baykal-Amur Railroad.

Of course, with regard to tularemia prevention attention is focused mainly on vaccination. Work in this direction is being constantly refined. Considering the existing system of measures for the prevention of tularemia, the USSR Ministry of Health issued an order on 5 February 1976, "On Measures for the Prevention of Tularemia Among the Public." This order approved the "Methodological Instructions for Planned Preventive Inoculations Against Tularemia," and described distinct concepts of territories enzootic for tularemia, procedure for detecting them, reporting and eliminating enzootics. The methodological instructions call for a differentiated approach to scheduling inoculations on the basis of exact determination of groups that are really exposed to the risk of F. tularensis infection. At the present time, within the limits of an administrative region deemed to be enzootic for tularemia, scheduled inoculations cover only the public residing or working in the enzootic territory of the village soviet with sites of the meadow-field, steppe, backwater-swamp or foothill-brook type, as well as inhabitants of adjacent village soviets if the landform and economic indicators of their territory do not differ from the rural soviet with established enzootic.

A definite approach has also been formed to planning of inoculations in accordance with epidemic activity of enzootic tularemia sites. For example, in sites of the meadow-field type, one does not have to immunize children up to 14 years of age, retired persons, the disabled, and there are expanded contraindications for immunization. In areas with inactive endemic sites, where only isolated cases of the disease had been recorded in the past, inoculations have become strictly occupational.

This order augmented appreciably the role of health ministries of Union republics, as well as local health agencies, in planning immunization of the public, since the question of declaring and eliminating enzootic nature of regions is within their competence.

Monitoring of immunization in the country and immunological structure of the population revealed that, in general, this work is being done correctly, and the immune stratum of public exposed to the risk of infection by F. tularensis constitutes about 85%, i.e., it meets the established requirements (Table 2).

It should be noted that the number of people immunized against tularemia in the USSR annually is quite sizable (Table 3), from 5,812,000 to 13,814,000 (in the period following adoption of scheduled mass scale vaccination, i.e., after 1949).

Prompt forecasts of the epizootic and epidemic situation in different regions is important to the prevention of group morbidity and proper organization of preventive measures. Sufficient attention is given to making them in all of the republics. Thus, epizootic complications pertaining to tularemia in West Siberia and the Ukraine in 1972 were forecast in good time. However, in cases where little zooparasitological work is being done, which is used